

For Reference

NOT TO BE TAKEN FROM THIS ROOM

Ex libris
UNIVERSITATIS
ALBERTAENSIS





Digitized by the Internet Archive
in 2019 with funding from
University of Alberta Libraries

<https://archive.org/details/McKim1977>

T H E U N I V E R S I T Y O F A L B E R T A

RELEASE FORM

NAME OF AUTHOR H. Robert McKim

TITLE OF THESIS..... The Effects of p-Chloroamphetamine with
..... and without a Tryptophan Load on the
.....
..... Regional Distribution of Dopamine,
.....
..... Noradrenaline and 5-Hydroxytryptamine
.....
..... in the Rat Brain.
.....

DEGREE FOR WHICH THESIS WAS PRESENTED Master of Science

YEAR THIS DEGREE WAS GRANTED 1977

Permission is hereby granted to THE UNIVERSITY OF
ALBERTA LIBRARY to reproduce single copies of this
thesis and to lend or sell such copies for private,
scholarly or scientific research purposes only.

The author reserves other publication rights, and
neither the thesis nor extensive extracts from it may
be printed or otherwise reproduced without the author's
written permission.

THE UNIVERSITY OF ALBERTA

THE EFFECTS OF p-CHLOROAMPHETAMINE WITH AND WITHOUT A TRYPTOPHAN LOAD
ON THE REGIONAL DISTRIBUTION OF
DOPAMINE, NORADRENALINE AND 5-HYDROXYTRYPTAMINE IN THE RAT BRAIN

by



H. ROBERT McKIM

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH
IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE
OF MASTER OF SCIENCE

IN

EXPERIMENTAL MEDICINE

DEPARTMENT OF MEDICINE

EDMONTON, ALBERTA

FALL, 1977

THE UNIVERSITY OF ALBERTA

FACULTY OF GRADUATE STUDIES AND RESEARCH

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research, for acceptance, a thesis entitled The Effects of p-Chloroamphetamine with and without a Tryptophan Load on the Regional Distribution of Dopamine, Noradrenaline and 5-Hydroxytryptamine in the Rat Brain submitted by H. Robert McKim in partial fulfilment of the requirements for the degree of Master of Science in Experimental Medicine.

ABSTRACT

In recent years a variety of amine hypotheses have been advanced to account for affective disorders. The amines implicated in these theories have included dopamine (DA), noradrenaline (NA) and serotonin (5HT). Amongst other evidence the effects on these amines of two types of antidepressants, namely the tricyclics and the monoamine oxidase inhibitors have been adduced to support these hypotheses. p-Chloroamphetamine (pCA) has also been shown to be an effective antidepressant in man. However, it is neither a tricyclic nor a monoamine oxidase inhibitor. Therefore it seemed that a study of the effects of this drug on the various amines listed above in different parts of the brain would shed further light on the biochemical mechanisms underlying affective disorders. Such a study is reported here.

pCA has recently been shown to have long term effects on the levels of 5HT and tryptophan hydroxylase in various species of animals. Recent histological examinations have shown that the drug has a neurotoxic action on a specific 5HT cell group in the mesencephalon of the rat. Other evidence suggests that the effects of pCA on the levels of 5HT and tryptophan hydroxylase are not due solely to this neurotoxic action in these serotonergic cell bodies. The study to be reported was designed to examine the effects of pCA on DA, NA and 5HT in areas which contained predominantly cell bodies and predominantly terminal concentrations of these amines.

The effects of a single injection of pCA (10 mg/kg) was assessed at both 3 and 30 days after injection. The effects of pCA on the

ability of the animals to utilize tryptophan to increase endogenous levels of 5HT was also assessed at both time periods. The levels of the various amines studied were analyzed using established spectrophotofluorometric techniques.

As predicted, pCA had no significant effect on the levels of DA or NA in any of the areas studied. On the other hand pCA had a marked effect on the levels of 5HT in all areas studied except the corpus striatum. The results obtained suggested a difference in action of pCA between brainstem areas and forebrain areas. Forebrain areas were unable to utilize a tryptophan load 3 days after pCA treatment but were able to do so 30 days after the pCA treatment. Brainstem areas were able to utilize a tryptophan load to increase endogenous levels of 5HT at both 3 and 30 days after pCA treatment.

The results suggest a differential action of pCA on the various areas studied. The importance of regional studies on brain tissue in elucidating the action of a drug is emphasized. Since this drug has no action on the catecholamines (DA and NA) its efficacy in the treatment of affective disorders does not support the catecholamine theory of these diseases.

ACKNOWLEDGEMENTS

I wish to express my sincere appreciation for the assistance and guidance given throughout this study by my supervisor Dr. W. G. Dewhurst.

For assistance in the analysis of the data I wish to thank Dr. R. W. Nutter.

I would also like to thank Mrs. Diane Calverley for all her assistance throughout this study.

To Mrs. Valerie Miller I wish to express my gratitude for her proficient typing.

Last, to my wife Pat, my thanks for her patience and understanding.

TABLE OF CONTENTS

Section	Page
1. Introduction	1
1.1 Distribution of DA, NA and 5HT in brain tissue	4
1.1.1 Biochemical distribution	5
1.1.1.1 Dopamine	5
1.1.1.2 Noradrenaline	7
1.1.1.3 5-Hydroxytryptamine	9
1.1.1.4 Summary	13
1.1.2 Histochemical distribution	13
1.1.2.1 Dopamine	14
1.1.2.2 Noradrenaline	16
1.1.2.3 5-Hydroxytryptamine	19
1.1.2.4 Summary	21
1.2 Metabolism of amines	21
1.2.1 Dopamine and noradrenaline	21
1.2.2 5-Hydroxytryptamine	26
1.3 Synaptic physiology of biogenic amines	29
1.4 Normal physiological role of DA, NA and 5HT in the CNS	29
1.4.1 Role of DA	29
1.4.2 Role of NA	34
1.4.3 Role of 5HT	36
1.5 Role of cerebral amines in pathology of mood	41
1.5.1 The biogenic amine theory of affective psychoses	41
1.6 Pharmacological and biochemical effects of pCA on amines	47

	Page
2. Design of this study	67
3. Materials and methods	68
3.1 Materials and instruments	68
3.2 Methods	68
3.2.1 Drug treatments	68
3.2.2 Dissection techniques	69
3.2.3 Biochemical techniques	72
3.2.3.1 Extraction procedures	72
3.2.3.2 Analysis of amines	73
3.2.4 Aminco Bowman Instrumentation	75
3.2.5 Calculations	76
4. Results	78
4.1 The effects after 3 and 30 days of tryptophan loading, pCA and pCA plus tryptophan on DA, NA and 5HT in the mesencephalon	79
4.1.1 Changes in DA levels	79
4.1.2 Changes in NA levels	79
4.1.3 Changes in 5HT levels	84
4.2 The effects of measuring amines at different times (3 or 30 days after treatment) on the levels of DA, NA and 5HT in the mesencephalon	87
4.2.1 Changes in DA levels	87
4.2.2 Changes in NA levels	89
4.2.3 Changes in 5HT levels	89
4.3 Results of analysis of variance done on the amounts of individual amines in the mesencephalon	90
4.4 Results of "t" tests done on individual groupings within the mesencephalon where significance was noted after the analysis of variance	90

	Page
4.5 The effects after 3 and 30 days of tryptophan loading, pCA and pCA plus tryptophan loading on DA, NA and 5HT in the diencephalon	96
4.5.1 Changes in DA levels	96
4.5.2 Changes in NA levels	99
4.5.3 Changes in 5HT levels	99
4.6 The effects of different times (3 or 30 days after treatment) on the levels of DA, NA and 5HT in the diencephalon	104
4.6.1 Changes in DA levels	104
4.6.2 Changes in NA levels	106
4.6.3 Changes in 5HT levels	107
4.7 Results of analysis of variance done on the amounts of individual amines in the diencephalon	107
4.8 Results of "t" tests done on individual groupings within the diencephalon where significance was noted after the analysis of variance	110
4.9 The effects after 3 and 30 days of tryptophan loading, pCA and pCA plus tryptophan loading on DA, NA and 5HT in the hippocampus	114
4.9.1 Changes in DA levels	114
4.9.2 Changes in NA levels	114
4.9.3 Changes in 5HT levels	117
4.10 The effects of different times (3 or 30 days after treatment) on the levels of NA and 5HT in the hippocampus	120
4.10.1 Changes in NA levels	120
4.10.2 Changes in 5HT levels	120
4.11 Results of analysis of variance done on the amounts of individual amines in the hippocampus	122
4.12 Results of "t" tests done on individual groupings within the hippocampus where significance was noted after the analysis of variance	125

	Page
4.13 The effects after 3 and 30 days of tryptophan loading, pCA and pCA plus tryptophan loading on DA, NA and 5HT in the corpus striatum	128
4.13.1 Changes in DA levels	128
4.13.2 Changes in NA levels	128
4.13.3 Changes in 5HT levels	133
4.14 The effects of different times (3 or 30 days after treatment) on the levels of DA, NA and 5HT in the corpus striatum	136
4.14.1 Changes in DA levels	136
4.14.2 Changes in NA levels	138
4.14.3 Changes in 5HT levels	138
4.15 Results of analysis of variance done on the amounts of individual amines in the corpus striatum	139
4.16 Results of "t" tests done on individual groupings within the corpus striatum where significance was noted after the analysis of variance	141
5. Discussion	148
6. Summary and conclusions	156
References	159
Appendix A: Chemicals	169
Appendix B: Equipment	170
Appendix C: Solutions	171
Appendix D: Levels of amines obtained from control animals after 3 days	172
Appendix E: Levels of amines obtained from control animals after 30 days	173
Appendix F: Levels obtained from tryptophan loaded animals after 3 days	174
Appendix G: Levels of amines obtained from tryptophan loaded animals after 30 days	175

	Page
Appendix H: Levels of amines obtained from pCA treated animals after 3 days	176
Appendix I: Levels of amines obtained from pCA treated animals after 30 days	177
Appendix J: Levels of amines obtained from pCA treated plus tryptophan loaded animals after 3 days	178
Appendix K: Levels of amines obtained from pCA treated plus tryptophan loaded animals after 30 days	179
Appendix L: Means and standard deviations in $\mu\text{g/gm}$ of DA in the different areas of the rat brain	180
Appendix M: Means and standard deviations in $\mu\text{g/gm}$ of NA in the different areas of the rat brain	181
Appendix N: Means and standard deviations in $\mu\text{g/gm}$ of 5HT in the different areas of the rat brain	182

LIST OF TABLES

Table	Description	Page
1	Regional distribution of DA in rat brain as reported by various authors.	6
2	Regional distribution of NA in rat brain as reported by various authors.	8
3	Regional distribution of 5HT in rat brain as reported by various authors.	11
4	Summary of effects of pCA on cerebral amines.	65
5	Schematic representation of the factorial design used in this experiment.	67
6	The percentage and direction of change for DA in the mesencephalon.	80
7	The percentage and direction of change for NA in the mesencephalon.	82
8	The percentage and direction of change for 5HT in the mesencephalon.	85
9	The percentage and direction of change for the differences in amine levels noted in the mesencephalon between animals measured 3 days after treatment and animals measured 30 days after treatment.	88
10	Results of analysis of variance done for DA in the mesencephalon.	91
11	Results of analysis of variance done for NA in the mesencephalon.	91
12	Results of analysis of variance done for 5HT in the mesencephalon.	92
13	Results of "t" tests for the levels of DA in animals who have been given pCA compared to those who have not been given pCA.	93
14	Results of "t" tests for the levels of DA comparing animals 3 days after treatment to animals 30 days after treatment.	93

Table	Description	Page
15	Results of "t" tests for the levels of 5HT in animals who have been given tryptophan compared to those who have not been given tryptophan.	94
16	Results of "t" tests for the levels of 5HT in animals who have been given pCA compared to those who have not been given pCA.	95
17	Results of "t" tests for the levels of 5HT comparing animals 3 days after treatment to animals 30 days after treatment.	95
18	The percentage and direction of change for DA levels in the diencephalon.	97
19	The percentage and direction of change for NA levels in the diencephalon.	100
20	The percentage and direction of change for 5HT levels in the diencephalon.	102
21	The percentage and direction of change for the differences in amine levels noted in the diencephalon between animals measured 3 days after treatment and animals measured 30 days after treatment.	105
22	Results of analysis of variance done for DA in the diencephalon.	108
23	Results of analysis of variance done for NA in the diencephalon.	108
24	Results of analysis of variance done for 5HT in the diencephalon.	109
25	Results of "t" tests for the levels of DA in animals who have been given tryptophan compared to those who have not been given tryptophan.	110
26	Results of "t" tests for the levels of 5HT in groups who have been given tryptophan compared to those who have not been given tryptophan.	111
27	Results of "t" tests for the levels of 5HT in animals who have been given pCA compared to those who have not been given pCA.	112
28	Results of "t" tests for the levels of 5HT comparing animals 3 days after treatment to animals 30 days after treatment.	112

Table	Description	Page
29	The percentage and direction of change for NA in the hippocampus.	115
30	The percentage and direction of change for 5HT in the hippocampus.	118
31	The percentage and direction of change for the differences in amine levels noted in the hippocampus between animals measured 3 days after treatment and animals measured 30 days after treatment.	121
32	Results of analysis of variance done for NA in the hippocampus.	123
33	Results of analysis of variance done for 5HT in the hippocampus.	123
34	Results of "t" tests for the levels of 5HT in animals who have been given tryptophan compared to those who have not been given tryptophan.	125
35	Results of "t" tests for the levels of 5HT in animals who have been given pCA compared to those who have not been given pCA.	126
36	Results of "t" tests for the levels of 5HT comparing animals 3 days after treatment to animals 30 days after treatment.	127
37	The percentage and direction of change for DA in the the corpus striatum.	129
38	The percentage and direction of change for NA in the corpus striatum.	131
39	The percentage and direction of change for 5HT in the corpus striatum.	134
40	The percentage and direction of change for the differences in amine levels noted in the corpus striatum between animals measured 3 days after treatment and animals measured 30 days after treatment.	137
41	Results of analysis of variance done for DA in the corpus striatum.	140
42	Results of analysis of variance done for NA in the corpus striatum.	140

Table	Description	Page
43	Results of analysis of variance done for 5HT in the corpus striatum.	142
44	Results of "t" tests for the levels of DA comparing animals 3 days after treatment to animals 30 days after treatment.	143
45	Results of "t" tests for the levels of NA in groups who have been given tryptophan compared to those who have not been given tryptophan.	144
46	Results of "t" tests for the levels of NA in groups who have been given pCA compared to those who have not been given pCA.	144
47	Results of "t" tests for the levels of NA comparing animals 3 days after treatment to animals 30 days after treatment.	145
48	Results of "t" tests for the levels of 5HT in groups who have not been given tryptophan.	145
49	Results of "t" tests for the levels of 5HT in animals who have been given pCA compared to those who have not been given pCA.	146
50	Results of "t" tests for the levels of 5HT comparing animals 3 days after treatment to animals 30 days after treatment.	146

LIST OF FIGURES

Figure	Description	Page
1	Diagram of a sagittal section (after Ungerstedt, 1971) showing DA cell groups in rat with their projections in relation to brain areas examined in the present study.	15
2	Diagram of a sagittal section (after Ungerstedt, 1971) showing NA cell groups in rat with their projections in relation to brain areas examined in the present study.	17
3	Diagram of a sagittal section (after Fuxe and Jonsson, 1974) showing 5HT cell groups in rat with their projections in relation to brain areas examined in the present study.	20
4	Diagrammatic representation of DA, NA and 5HT fibre tracts originating from the brain stem (adapted from Anden et al. 1966).	22
5	Synthesis and degradation of Dopamine.	23
6	Synthesis and degradation of Noradrenaline.	24
7	Synthesis and degradation of 5-Hydroxytryptamine.	27
8	Schematic diagram showing processes at the monoaminergic synaptic level in CNS.	30
9	Mean values of DA levels in mesencephalon showing the effects of tryptophan loading (try), pCA treatment (pCA) and a combined treatment (pCA + try).	81
10	Mean values of NA levels in the mesencephalon showing the effects of tryptophan loading (try), pCA treatment (pCA) and a combined treatment (pCA + try).	83
11	Mean values of 5HT levels in mesencephalon showing the effects of tryptophan loading (try), pCA treatment (pCA) and a combined treatment (pCA + try).	86
12	Mean values of DA levels in diencephalon showing the effects of tryptophan loading (try), pCA treatment (pCA) and a combined treatment (pCA + try).	98

Figure	Description	Page
13	Mean values of NA levels in diencephalon showing the effects of tryptophan loading (try), pCA treatment (pCA) and a combined treatment (pCA + try).	101
14	Mean values of 5HT levels in diencephalon showing the effects of tryptophan loading (try), pCA treatment (pCA) and a combined treatment (pCA + try).	103
15	Mean values of NA levels in hippocampus showing the effects of tryptophan loading (try), pCA treatment (pCA) and a combined treatment (pCA + try).	116
16	Mean values of 5HT levels in hippocampus showing the effects of tryptophan loading (try), pCA treatment (pCA) and a combined treatment (pCA + try).	119
17	The effects of tryptophan loading on the levels of 5HT in the hippocampus in animals without previous pCA treatment or in animals treated previously with pCA.	124
18	The effects of tryptophan loading on the levels of 5HT in the hippocampus at both 3 days and 30 days after treatment.	125
19	Mean values of DA in corpus striatum showing the effects of tryptophan loading (try), pCA treatment (pCA) and a combined treatment (pCA + try).	130
20	Mean values of NA in corpus striatum showing the effects of tryptophan loading (try), pCA treatment (pCA) and a combined treatment (pCA + try).	132
21	Mean values of 5HT in corpus striatum showing the effects of tryptophan loading (try), pCA treatment (pCA) and a combined treatment (pCA + try).	135
22	The effects of a tryptophan load on the levels of NA in each of the experimental conditions.	141

(1.) INTRODUCTION

Since Twarog and Page established the presence of 5-hydroxy-tryptamine (5HT) in the central nervous system (CNS) in 1953 and Vogt in 1954 described the distribution of noradrenaline (NA) in the reticular system, the functions of these cerebral amines have been the subject of extensive research and lively debate. Shortly thereafter, Montague (1957) found dopamine (DA) to be a normal constituent of CNS tissues and a description of the role of this amine has been extensively pursued. More recently (1972-1975) the presence of unhydroxylated amines (tryptamine and phenylethylamine) has been firmly established in CNS tissues (Boulton, 1976) and the physiological role of these amines is presently the subject of much stimulating research. These amines have been collectively referred to in the literature as biogenic amines. 5HT, NA and DA, like acetylcholine (ACh), are considered to be classical neurotransmitters; tryptamine and phenylethylamine along with others whose concentrations are in the nanogram per gram range have been referred to as trace amines. Whether these trace amines exist as transmitters in the classical sense is at present a debated issue (Green and Grahame-Smith, 1975).

The roles of 5HT and NA have, in many cases, been considered together in discussion of the integration of different physiological systems in the CNS. If 5HT activates a particular system then NA inhibits it or vice versa. DA appeared, at first to be involved in physiological systems which are independent of the other two biogenic amines. More recent work on the other hand has incorporated this

amine into a total picture with either 5HT or NA or both in the description of various physiological processes. Both NA and 5HT have been described as neurotransmitters or neuroregulators involved in various physiological processes including sleep (Jouvet, 1974), thermoregulation, (Marley and Stephenson, 1972), sexual behavior (Gessa and Tagliamonte, 1974), pain sensitivity (Barchas, 1972) and arousal (Mandel and Segal, 1973). DA has been implicated in motor activity (Barbeau, 1972), stereotyped behavior (Bieger and Hockman, 1975), autonomic control through the striatum (Barbeau, 1972), hormonal release (Donovan, 1970) and control of metabolism of NA and 5HT in the striatum and other areas of the CNS (Bieger and Hockman, 1975).

All three of these amines have been implicated in the pathology of the affective disorders. Pharmacological evidence suggests that drugs which relieve depression do so by increasing one or another of the biogenic amines at receptor sites in the brain while drugs that reverse mania do so by decreasing the action of one or another of the biogenic amines at receptor sites in the brain (Schildkraut, 1974). Various groups have held different theories with respect to the causative agents in the etiology of the disease state (Schildkraut, 1965; Lapin and Oxenkrug, 1969; Dewhurst, 1968). To date, none of the theories implicating the classical neurotransmitters have held up under critical examination. The only good evidence for involvement of an amine modulator of mood in man, is that involvement reputed for the unhydroxylated amines, tryptamine and phenylethylamine (Dewhurst, 1968). The fact that these amines do not appear to exist as neurotransmitters in the classical sense does not refute the

evidence for the potency of these amines on animal behavior (Dewhurst and Marley, 1965). If these amines cannot be defined as neurotransmitters in the classical sense then possibly it is time to reassess the criteria necessary for a compound to be defined as a neurotransmitter, or alternatively consider some other means by which these amines may affect the classical neurotransmitters in their involvement in the regulation of mood.

Current trends in antidepressant drug therapy involve the use of relatively non-specific drugs. These compounds are classed into two different groups: the tricyclic antidepressants which act on the neuronal membrane by effectively shutting down the re-uptake systems and the monoamine oxidase inhibitors which prevent the metabolic action of monoamine oxidase on transmitter molecules (Goodman and Gilman, 1970). Both of these groups reputedly act by increasing the concentration of transmitter molecules at the synapse thus potentiating their action. The introduction of compounds, into clinical use, which selectively enhance or inhibit particular monoamines is required to further evaluate the roles of particular neurotransmitters in depression.

p-Chloroamphetamine (pCA) an unhydroxylated amine has been shown to reduce the level of 5-hydroxytryptamine (5HT) in brain tissue (Fuller et al., 1965). Recent reports on the efficacy of pCA in the treatment of depression have been favorable (Van Praag et al., 1971) and its effects on 5HT and 5-hydroxyindole acetic acid (5HIAA) excretion in humans (Van Praag et al., 1970) indicate the importance of elucidating the mechanism of action of this compound. However of greater importance to further clinical trials is the recent report of

Harvey et al. (1975) showing that pCA may have a neurotoxic effect on brain tissue. These authors have found that a single injection of this drug in rats results in neuronal destruction in as little as one day with the effect lasting for at least 30 days.

The purpose of this experiment is to elucidate in rats the effects of pCA on the levels of three classical neurotransmitters DA, NA and 5HT at 3 and 30 days after pCA treatment. Because this compound is reputed to be a tryptophan hydroxylase blocker (Sanders-Bush, 1970) the animals ability to metabolize a tryptophan load will also be assessed 3 and 30 days after pCA treatment. These effects will be analyzed in various areas of the brain to determine if there are regional differences in the actions of this drug.

This thesis will cover a selective review of the literature which is pertinent to this work. This will be followed by a discussion of the experimental work and results. The final section will be devoted to the discussion of these results and suggestions for future experiments.

(1.1) Distribution of DA, NA and 5HT in brain tissue.

The existence of DA, NA and 5HT as normal constituents of the central nervous system has long been accepted. The distribution of these amines has been assessed using biochemical and histochemical procedures. The agreement between these two types of analyses is, in most cases, excellent. Biochemically, analysis is conducted on extractions of whole brain or discrete areas of the central nervous system. Histochemical and morphological distributions are assessed in tissue sections.

(1.1.1) Biochemical Distribution

With the first reports of the presence of NA (Vogt, 1954) and DA (Montagu, 1957) in CNS tissues much interest has been generated in establishing the distribution of these monoamines in the brain. Until recently, methods for the analysis of these compounds have lacked sufficient sensitivity and specificity to measure other than each of the amines separately in whole brain samples. The introduction of the fluorescence technique of Chang (1964) and subsequent improvements by Laverty and Taylor (1968) have allowed for analyses to be done on discrete areas in the brain. Likewise, these newer techniques allow for the estimation of both catecholamines in a single sample.

(1.1.1.1) Dopamine

Table 1 presents the levels of dopamine reported in some of the more recent literature. Agreement is not consistent but patterns of regional variations are unquestionable.

The highest concentration of dopamine is found in the corpus striatum. The majority of the values reported are around $6.5 \mu\text{g/gm}$ (Shellenberger and Gordon, 1971; Butterworth et al., 1975). One value, $3.55 \pm .398 \mu\text{g/gm}$ (Haubrich and Denzer, 1973) is much lower. The region with the next highest concentration is the hypothalamus where levels are reported to be around $.30 \mu\text{g/gm}$ (Haubrich and Denzer, 1973; Butterworth et al., 1975). The midbrain which usually includes the hypothalamus in most dissection techniques, contains levels around $.20 \mu\text{g/gm}$ (Shellenberger and Gordon, 1971; Cox and Perhach, 1973; Butterworth et al., 1975). In all other areas of the rat brain except possibly the cerebellum the levels reported showed considerable

Table 1 Regional distribution of DA in rat brain as reported by various authors.

<u>Region</u>	<u>ug/gm</u>	<u>Source</u>
Whole brain	1.06 ± .07	1
Whole brain	.843 ± .016	7
Whole brain	.55 ± .04	9
Cerebral cortex	1.12 ± .049	3
Cerebral cortex	.004 ± .008	5
Cerebral cortex	.138 ± .015	6
Cerebral cortex	1.248 ± .042	7
Cerebral cortex	.33 ± .03	9
Cerebellum	.029 ± .011	3
Cerebellum	.003 ± .004	5
Cerebellum	.029 ± .009	7
Cerebellum	.03 ± .02	9
Striatum	7.112 ± 1.62	5
Striatum	3.55 ± .398	6
Striatum	6.33 ± .56	9
Hippocampus	.07 ± .03	9
Hypothalamus	.306 ± .077	6
Hypothalamus	.27 ± .12	9
Midbrain	.16 ± .05	5
Midbrain	.242 ± .016	7
Midbrain	.19 ± .02	9
Medulla & pons	.090 ± .012	3
Medulla & pons	.024 ± .012	5
Medulla & pons	.260 ± .168	6
Medulla & pons	.063 ± .017	7
Medulla & pons	.04 ± .02	9

Sources:

1. Ansell and Beeson, 1968
2. Maickel et al., 1968
3. Kariya and Aprison, 1969
4. Miller et al., 1970
5. Shellenberger and Gordon, 1971
6. Haubrich and Denzer, 1973
7. Cox and Perhach, 1973
8. Saavedra et al., 1973
9. Butterworth et al., 1975

variation. For the cerebellum levels reported by three authors are consistent at $.03 \mu\text{g/gm}$ (Kariya and Aprison, 1969; Cox and Perhach, 1973; Butterworth, et al., 1975). One author, however, reports a level of $.003 \mu\text{g/gm}$ (Shellenberger and Gordon, 1971). Levels reported for the cerebral cortex show considerable variation which is undoubtedly due to the inclusion of the striatum in this area by some authors (Kariya and Aprison, 1969; Cox and Perhach, 1973). Even whole brain levels vary from $.55 \pm .04 \mu\text{g/gm}$ to $1.06 \pm .07 \mu\text{g/gm}$ (Ansell and Beeson, 1968; Cox and Perhach, 1975; Butterworth et al., 1975).

(1.1.1.2) Noradrenaline

Table 2 presents the levels reported by various authors for the regional distribution of NA in the rat brain. Levels reported for NA by different workers are much more consistent than are those reported for DA and 5HT.

The highest concentration of NA is found in the hypothalamus. The majority of the values reported are around $1.0 \mu\text{g/gm}$ (Maickel et al., 1968; Miller et al., 1970; Haubrich and Denzer, 1973). The area with the next highest concentration is the midbrain where values reported are consistently around $.65 \mu\text{g/gm}$ (Maickel et al., 1968; Miller et al., 1970; Shellenberger and Gordon, 1971; Cox and Perhach, 1973). Only one value falls outside this region that being $.39 \pm .02 \mu\text{g/gm}$ as reported by Butterworth et al. (1970). In the medulla pons or medulla alone two different values are commonly reported, $.50 \mu\text{g/gm}$ (Miller et al., 1970; Shellenberger and Gordon, 1971; Cox and Perhach, 1973; Butterworth et al., 1975) and $.75 \mu\text{g/gm}$ (Maickel et al., 1968; Kariya and Aprison, 1969; Haubrich and Denzer, 1973). Levels in the

Table 2 Regional distribution of NA in rat brain as reported by various authors.

<u>Region</u>	<u>μg/gm</u>	<u>Source</u>
Whole brain	.40 ± .08	1
Whole brain	.44 ± .06	2
Whole brain	.38 ± .016	7
Whole brain	.31 ± .02	9
Cerebral cortex	.30 ± .03	2
Cerebral cortex	.42 ± .03	4
Cerebral cortex	.269 ± .84	5
Cerebral cortex	.169 ± .107	6
Cerebral cortex	.281 ± .016	7
Cerebral cortex	.22 ± .02	9
Cerebellum	.18 ± .02	2
Cerebellum	.339 ± .057	3
Cerebellum	.21 ± .02	4
Cerebellum	.192 ± .046	5
Cerebellum	.270 ± .017	6
Cerebellum	.190 ± .018	7
Cerebellum	.17 ± .03	9
Striatum	.300 ± .091	5
Striatum	.203 ± .034	6
Striatum	.22 ± .04	9
Hippocampus	.355 ± .017	6
Hippocampus	.21 ± .03	9
Hypothalamus & thalamus	1.11 ± .08	2
Hypothalamus	1.24 ± .24	4
Hypothalamus	.997 ± .085	6
Hypothalamus	1.52 ± .17	9
Midbrain, thalamus & subthalamus	.372 ± .017	6
Midbrain	.65 ± .14	2
Midbrain	.64 ± .06	4
Midbrain	.66 ± .163	5
Midbrain	.607 ± .028	7
Midbrain	.39 ± .02	9
Medulla & pons	.738 ± .096	3
Medulla & pons	.510 ± .131	5
Medulla & pons	.913 ± .034	6
Medulla & pons	.585 ± .022	7
Medulla & pons	.48 ± .04	9
Medulla	.74 ± .12	2
Medulla	.56 ± .05	4

Sources:

1. Ansell and Beeson, 1968
2. Maickel et al., 1968
3. Kariya and Aprison, 1969
4. Miller et al., 1970
5. Shellenberger and Gordon, 1971
6. Haubrich and Denzer, 1973
7. Cox and Perhach, 1973
8. Saavedra et al., 1973
9. Butterworth et al., 1975

hippocampus are around $.30 \mu\text{g/gm}$ (Haubrich and Denzer, 1973; Butterworth et al., 1975) and in the striatum are around $.20 \mu\text{g/gm}$ (Shellenberger and Gordon, 1971; Haubrich and Denzer, 1973; Butterworth et al., 1975). Concentrations of NA in the cerebral cortex and the cerebellum are consistent at $.20 - .25 \mu\text{g/gm}$ with only a few deviations. For the cerebral cortex Kariya and Aprison (1969) report levels of $.459 \pm .054 \mu\text{g/gm}$, and Miller et al. (1970) report $.42 \pm .03 \mu\text{g/gm}$. Whole brain values are reported to be around $.40 \mu\text{g/gm}$ (Ansell and Beeson, 1968; Maickel et al., 1968; Cox and Perhach, 1973).

(1.1.1.3) 5-hydroxytryptamine

Ever since Twarog and Page (1953) first reported that 5HT was present in brain tissues, the distribution of this amine in cerebral tissues has been extensively studied. Early workers were hindered in the analysis of 5HT by the inability of their techniques to measure the low concentrations in brain tissue. In 1965 Snyder et al. introduced a method to improve the sensitivity of 5HT assays of central nervous system tissue. The method was based on the generation of a fluorophore from 5HT by a reaction with ninhydrin. The technique was limited though in that the reaction lacked specificity and as was later pointed out, ninhydrin reacts with many drugs which are often used in 5HT studies. Later, Maickel et al. (1968) described another technique for increasing the sensitivity of 5HT measures. This technique which is based on the generation of a fluorophore from a reaction of 5HT with o-phthalaldehyde improves the specificity of the method since it only reacts with 5 substituted indoles, and it does not react with drugs as does ninhydrin. By improving the sensitivity

and specificity of analysis for 5HT these two methods have allowed for analysis to be conducted on much smaller tissue samples.

Consequently, many workers have now mapped the differential distribution of this amine in central nervous system tissue.

At the present there is little agreement as to the actual quantities of 5HT in each of the areas in the brain. Table 3 presents 5HT levels from some of the most recent literature dating back to the introduction of the o-phthalaldehyde and ninhydrin reactions. Most of the results reported are based on fluorometric analysis, with the exception of a radioisotope technique (Saavedra et al., 1973).

The highest concentration of 5HT is found in the midbrain area. Values reported are most often around 1 $\mu\text{g/gm}$ (Maickel et al., 1968; Miller et al., 1970; Curzon and Green, 1970; Shellenberger and Gordon, 1971; Saavedra et al., 1973). Two lower values of $.605 \pm .063$ $\mu\text{g/gm}$ (Cox and Perhach, 1973) and $.67 \pm .10$ $\mu\text{g/gm}$ (Butterworth et al., 1975) have also been reported. Values for the hypothalamus, when it is reported separately are similar to those for the midbrain (Maickel et al., 1968; Miller et al., 1970; Haubrich and Denzer, 1973; Saavedra et al., 1973; Butterworth et al., 1975) except for those values reported by Curzon and Green (1970). Their values for the hypothalamus are two times greater than that for the midbrain, being $2.65 \pm .26$ $\mu\text{g/gm}$ and $1.03 \pm .01$ $\mu\text{g/gm}$ respectively. The next highest concentration of 5HT is found within the medulla-pons area. The majority of the values reported for this area are around .80 $\mu\text{g/gm}$ (Shellenberger and Gordon, 1971; Haubrich and Denzer, 1973; Cox and Perhach, 1973; Saavedra et al., 1973). Two values are somewhat lower with

Table 3 Regional distribution of 5HT in rat brain as reported by various authors.

<u>Region</u>	<u>µg/gm</u>	<u>Source</u>
Whole brain	.54 ± .04	1
Whole brain	.55 ± .07	4
Whole brain	.83 ±	5
Whole brain	.464 ± .032	8
Whole brain	.62 ± .04	9
Whole brain	.45 ± .03	10
Cerebral cortex	.35 ± .04	2
Cerebral cortex	.33 ± .055	3
Cerebral cortex	.65 ± .05	4
Cerebral cortex	.52 ± .03	5
Cerebral cortex	.358 ± .06	6
Cerebral cortex	.282 ± .018	7
Cerebral cortex	.417 ± .019	8
Cerebral cortex	.57 ± .062	9
Cerebral cortex	.26 ± .02	10
Cerebellum	.24 ± .03	2
Cerebellum	.052 ± .009	3
Cerebellum	.19 ± .01	4
Cerebellum	.39 ± .08	5
Cerebellum	.156 ± .06	6
Cerebellum	.150 ± .018	7
Cerebellum	.078 ± .015	8
Cerebellum	.07 ± .008	9
Cerebellum	.09 ± .01	10
Striatum	1.36 ± .23	5
Striatum	.657 ± .184	6
Striatum	.493 ± .035	7
Striatum	.57 ± .083	9
Striatum	.52 ± .09	10
Hippocampus	.493 ± .018	6
Hippocampus	.53 ± .02	8
Hippocampus	.44 ± .08	9
Hypothalamus & thalamus	1.29 ± .15	2
Hypothalamus	.97 ± .11	4
Hypothalamus	2.64 ± .26	5
Hypothalamus	.862 ± .053	7
Hypothalamus	.98 ± .051	9
Hypothalamus	.95 ± .10	10
Midbrain, thalamus & subthalamus	.528 ± .018	7
Midbrain	1.23 ± .16	2
Midbrain	1.06 ± .08	4
Midbrain	1.03 ± .10	5
Midbrain	.941 ± .13	6
Midbrain	.605 ± .063	8
Midbrain	.97 ± .035	9
Midbrain	.67 ± .10	10
Medulla & pons	.468 ± .057	3
Medulla & pons	1.24 ± .10	5
Medulla & pons	.665 ± .132	6
Medulla & pons	.801 ± .053	7
Medulla & pons	.882 ± .057	8
Medulla & pons	.83 ± .09	9
Medulla & pons	.50 ± .06	10
Medulla	1.03 ± .12	2
Medulla	.57 ± .07	4

Sources:

1. Ansell and Beeson, 1968
2. Maickel et al., 1968
3. Kariya and Aprison, 1969
4. Miller et al., 1970
5. Curzon and Green, 1970
6. Shellenberger and Gordon, 1971
7. Haubrich and Denzer, 1973
8. Cox and Perhach, 1973
9. Saavedra et al., 1973
10. Butterworth et al., 1975

Butterworth et al. (1975) reporting $.50 \pm .06 \mu\text{g/gm}$ and Kariya and Aprison (1969) reporting $.468 \pm .057 \mu\text{g/gm}$. One considerably higher value of $1.2 \pm .01 \mu\text{g/gm}$ was reported by Curzon and Green, 1970. The next highest concentrations of 5HT are within the striatum and the hippocampus. In both areas the majority of the values reported are around $.5 \mu\text{g/gm}$ (Shellenberger and Gordon, 1971; Haubrich and Denzer, 1973; Saavedra et al., 1973; Butterworth et al., 1975). One value for the striatum of $1.36 \pm .26 \mu\text{g/gm}$ (Curzon and Green, 1970) is almost three times that reported by the others for this region. Both the cerebral cortex and the cerebellum contain minimal amounts of 5HT. In the cerebral cortex the values lie around $.30 \mu\text{g/gm}$ (Maickel et al., 1968; Kariya and Aprison, 1969; Shellenberger and Gordon, 1971). Four higher values have also been reported: $.65 \pm .05 \mu\text{g/gm}$ (Miller et al., 1970), $.57 \pm .062 \mu\text{g/gm}$ (Saavedra et al., 1973), $.52 \pm .03 \mu\text{g/gm}$ (Curzon and Green, 1970), and $.417 \pm .019 \mu\text{g/gm}$ (Cox and Perhach, 1973). In the cerebellum the majority of the reported values are about $.1 \mu\text{g/gm}$ (Kariya and Aprison, 1969; Shellenberger and Gordon, 1971; Haubrich and Denzer, 1973; Cox and Perhach, 1973; Saavedra et al., 1973; Butterworth et al., 1975). Three values are somewhat higher with two lying close to $.2 \mu\text{g/gm}$ (Maickel et al., 1968; Miller et al., 1970) and one of $.4 \mu\text{g/gm}$ (Curzon and Green, 1970). Whole brain levels of 5HT are reported to be between $.45 \pm .03 \mu\text{g/gm}$ and $.62 \pm .04 \mu\text{g/gm}$ (Ansell and Beeson, 1968; Maickel et al., 1968; Cox and Perhach, 1973; Saavedra et al., 1973; Butterworth et al., 1975). Whole brain estimates do not reflect the regional distribution of 5HT because there is an uneven distribution of this amine in different areas of the brain.

(1.1.1.4) Summary

The data can be summarized as follows:

1. Dopamine concentrations

striatum > hypothalamus > midbrain ? rest

2. Noradrenaline concentrations

hypothalamus > midbrain > medulla pons > hippocampus >

striatum = cortical tissues

3. 5HT concentrations

midbrain = hypothalamus > medulla > striatum =

hippocampus > cerebral cortex > cerebellum

Of interest is the similarity between the distributions of NA and 5HT and the dissimilarity of DA. More recent anatomical data described by Lindvall and Bjorklund (1974) shows much wider distribution of DA than has been previously indicated (Ungerstedt, 1971). With this morphological description the biochemical data as presented above appears erroneous. This suggests that present techniques for measuring DA biochemically, in brain regions are possibly suspect.

(1.1.2) Histochemical Distribution

By the application of the Falck Hillarp fluorescence histochemical technique (Carlsson et al., 1962; Falck et al., 1962) direct evidence for the cellular localization of monoamines in the central nervous system has been established. Using this technique which relies on the reaction of endogenous amines with formaldehyde gas and modifications of this technique, DA, NA and 5HT have been

extensively mapped in rat brain tissues.

Under the normal conditions of the formaldehyde technique, the fluorophore produced from 5HT, a β -carboline appears yellow under the fluorescence microscope and is only weakly visible. To improve this visualization monoamine oxidase inhibitors are used so that greater concentrations of the amine are present for reaction. Conversely, the product formed from the catecholamines, an isoquinoline appears green under the fluorescence microscope and is highly stable and can be easily visualized after the formaldehyde reaction.

Tools employed in mapping monoaminergic neuronal systems include lesioning experiments. The origin and destination of the various monoaminergic neurons can be determined by monitoring the degeneration in the distal portions of the lesioned axon and the eventual destruction in the originating cell bodies. Although not fully elucidated, much is known about the anatomical distribution of the various monoaminergic systems in the brain.

(1.1.2.1) Dopamine

There are two principle ascending dopaminergic fibre tracts, the nigrostriatal and mesolimbic systems (Anden et al., 1966; Understedt, 1971). See Fig. 1 . Both originate from a group of catecholaminergic cell bodies A8, A9 and A10, situated in the mesencephalon (Dahlstrom and Fuxe, 1965).

The nigrostriatal pathway, which arises from within the pars compacta of the substantia nigra (A9) ascends along a tract just dorsolateral of the medial forebrain bundle (MFB). This tract runs through the lateral hypothalamus into the internal capsule from

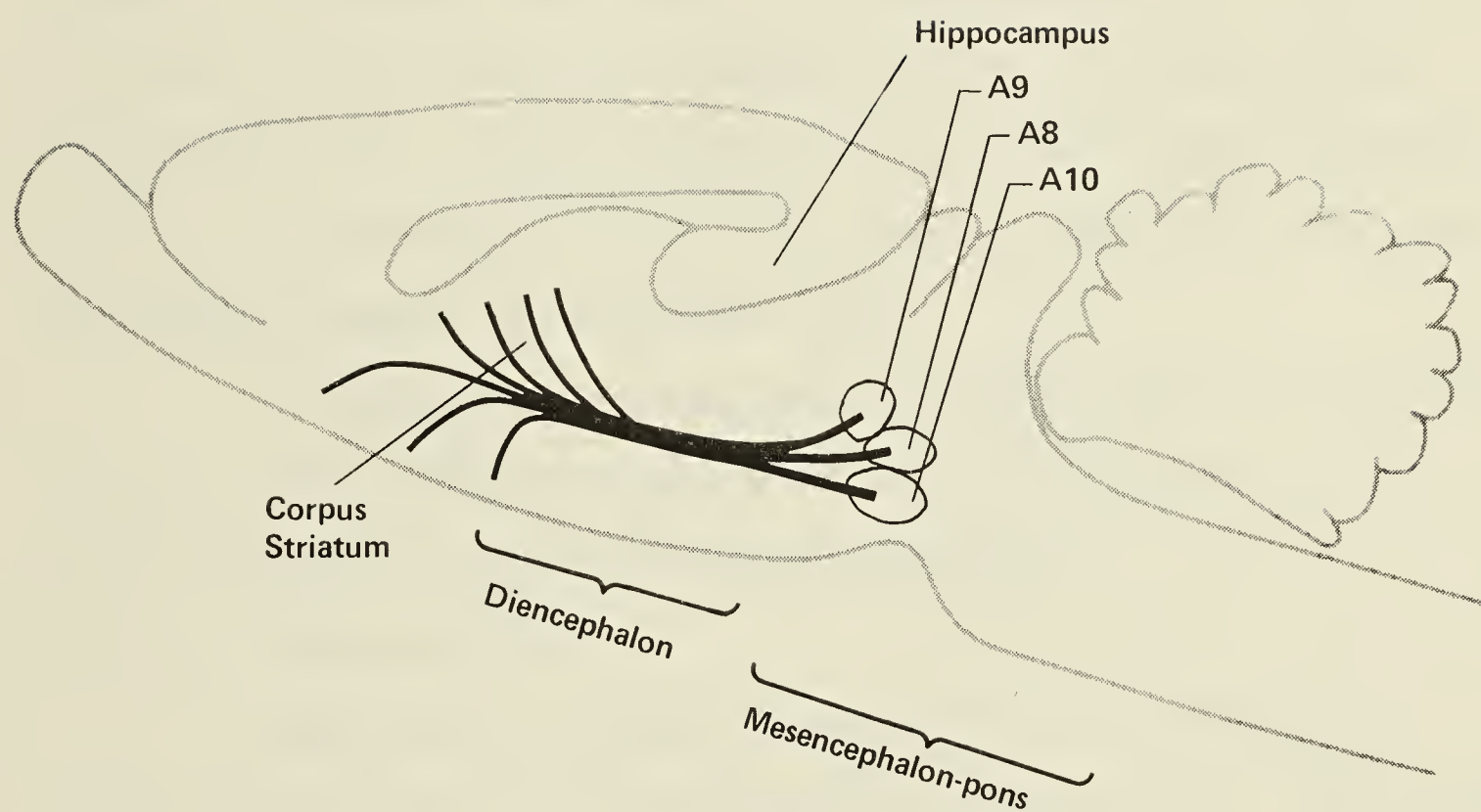


Fig. 1 Diagram of a sagittal section (after Ungerstedt, 1971) showing DA cell groups in rat with their projections in relation to brain areas examined in the present study.

- A8 = undefined, situated in reticular formation,
- A9 = substantia nigra,
- A10 = undefined, situated dorsal to interpeduncular nucleus

which it enters into the globus pallidus running straight through into the corpus striatum (Ungerstedt, 1971).

The mesolimbic system arises from a group of cell bodies which are just dorsolateral of the interpeduncular nucleus (A10). As this system ascends, along its path projections leave to enter the amygdala and the pyriform cortex, eventually reaching its rostral extensions within the nucleus accumbens and the olfactory tubercle (Ungerstedt, 1971). Extensions beyond these areas have been described with fibres projecting into the frontal cortex, the limbic cortex and the septum (Lundvall and Bjorklund, 1974).

A third major DA system has been found in the rat hypothalamus. The cells bodies (A12) have been described as the nucleus arcuatus, with its projects innervating the median eminence.

(1.1.2.2) Noradrenaline

Noradrenergic pathways originate from well defined cell bodies in the lower brain stem (Dahlstrom and Fuxe, 1965). They ascend along two major pathways, the dorsal tegmental bundle and the central tegmental tract (Ungerstedt, 1971; Lundvall and Bjorklund, 1974). See Fig. 2 . The central tegmental tract which arises from cell bodies located in the medulla oblongata and pons runs along an area closely associated with the pontine and mesencephalic tegmentum. The dorsal tegmental bundle on the other hand originates in total from the locus coeruleus (Lundvall and Bjorklund, 1974).

The dorsal tegmental bundle runs in a ventrorostral direction from the locus coeruleus towards the mid hypothalamus where it merges with the MFB. This tract eventually passes into the forebrain

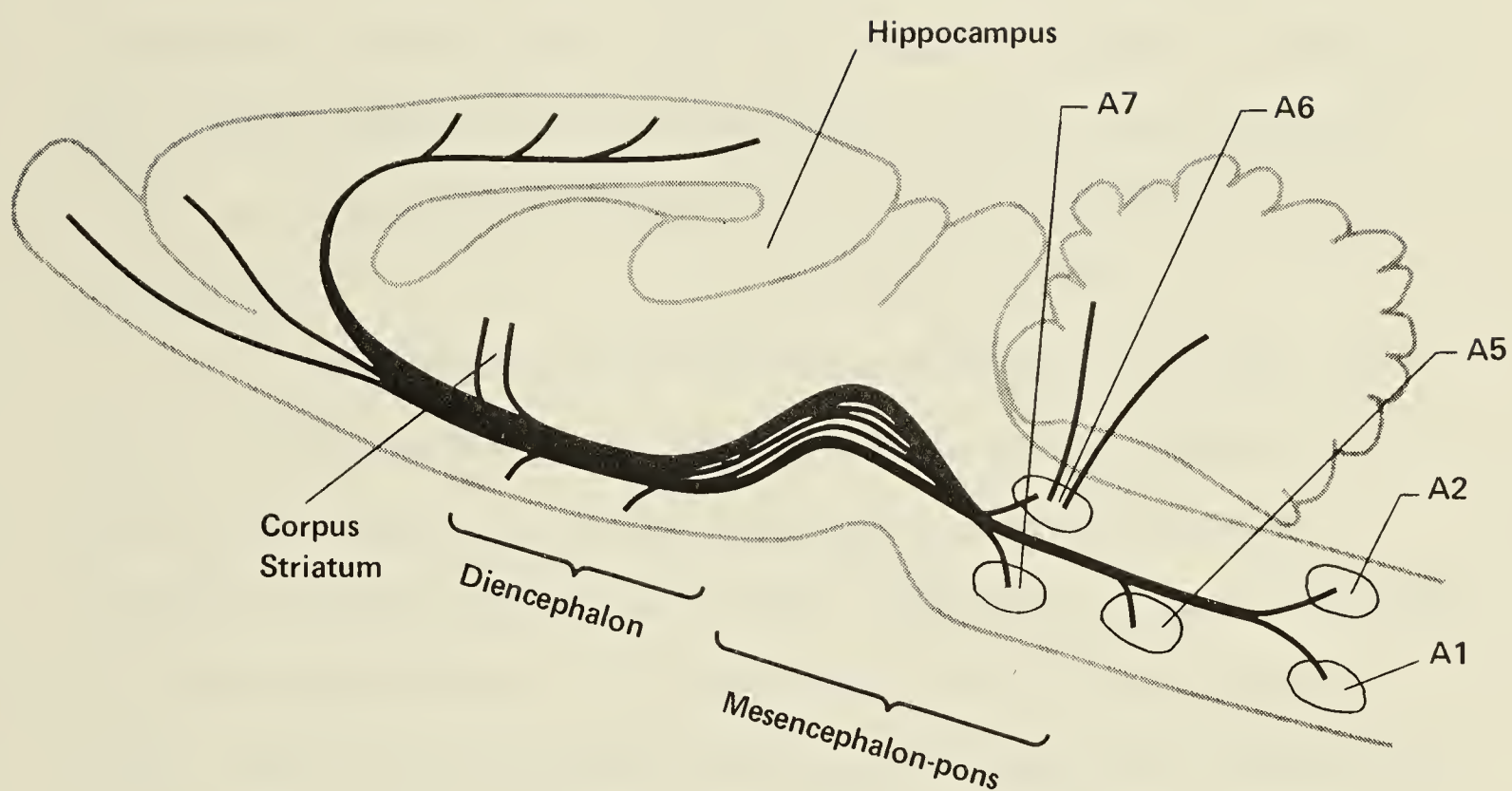


Fig. 2 Diagram of a sagittal section (after Ungerstedt, 1971) showing NA cell groups in rat with their projections in relation to brain areas examined in the present study.

- A1 = lateral reticular nucleus, A2 = nucleus commissuralis
 A5 = undefined, situated with fibers of the rubrospinalis
 A6 = locus coeruleus, A7 = subcoeruleus

through the internal capsule.

The central tegmental tract originates in the medulla oblongata and can be mapped along its full extension into the internal capsule. The tract ascends as a dense bundle travelling through the pons and passes into the mesencephalon. All of the major cell groups within this area contribute fibres to this tract. At the meso-diencephalic junction this tract contributes to the MFB. Eventually the central tegmental tract intermingles with the dorsal tegmental bundle as they both pass through the internal capsule into the fore-brain areas.

Those fibres which originate in the locus coeruleus and reach the MFB via the dorsal tegmental bundle are described as locus type fibre. Non-locus fibres then originate outside the locus coeruleus in other NA cell body areas. Both fibre systems contribute to a rich innervation in the hypothalamic, thalamic and preoptic areas. Locus type fibres but not non-locus type fibres project into the amygdala and through the fornix into the hippocampus. The caudate nucleus is also innervated from this system. At the rostral end of the septum a major portion of the tract turns in a dorsomedial direction and travels into the area of the corpus callosum. This branch runs above the corpus callosum and gives off fibres into the neocortex. It continues caudally into the hippocampus (Lundvall and Bjorklund, 1974). Non-locus fibres have been found to project into the amygdala and corpus striatum. Extensions beyond the preoptic and septal regions have not been described (Lundvall and Bjorklund, 1974).

(1.1.2.3) 5-hydroxytryptamine

The serotonergic system is situated medially in the rat brainstem. Cell bodies have been found in the raphe system of the medulla oblongata, pons and mesencephalon.

Both ascending and descending axons have been shown to originate from cell body areas (Dahlstrom and Fuxe, 1964; Fuxe and Jonsson, 1974; Fig. 3). Descending pathways originating from the medulla oblongata have been found to travel into the spinal cord (Dahlstrom and Fuxe, 1965). Ascending pathways arise mainly from cell groups in the caudal mesencephalon (groups B7, B8 and B9 according to Dahlstrom and Fuxe, 1964). Most of these ascending axons turn ventrally after leaving the cell bodies then rostrally towards the interpeduncular nucleus. Here they become the most ventral part of the MFB. They can be separated into a medial and lateral component. The lateral component originates from the dorsal (B7) and medial (B8) raphe nuclei. At its most rostral extension it joins the tractus diagonalis and after running dorsally in this tract it enters the cingulum to finally innervate the cortex and possibly the hippocampus (Fuxe and Jonsson, 1974). The medial pathway originates primarily from the mesencephalic and pontine nuclei and innervates the hypothalamus and preoptic areas. A far lateral tract has also been described which originates from the raphe nuclei of the mesencephalon and the B9 cell group which is located in the ventromedial reticular formation in close association with the B8 cell group. Its projections are found mainly within the corpus striatum (Anden et al., 1966; Fuxe and Jonsson, 1974).

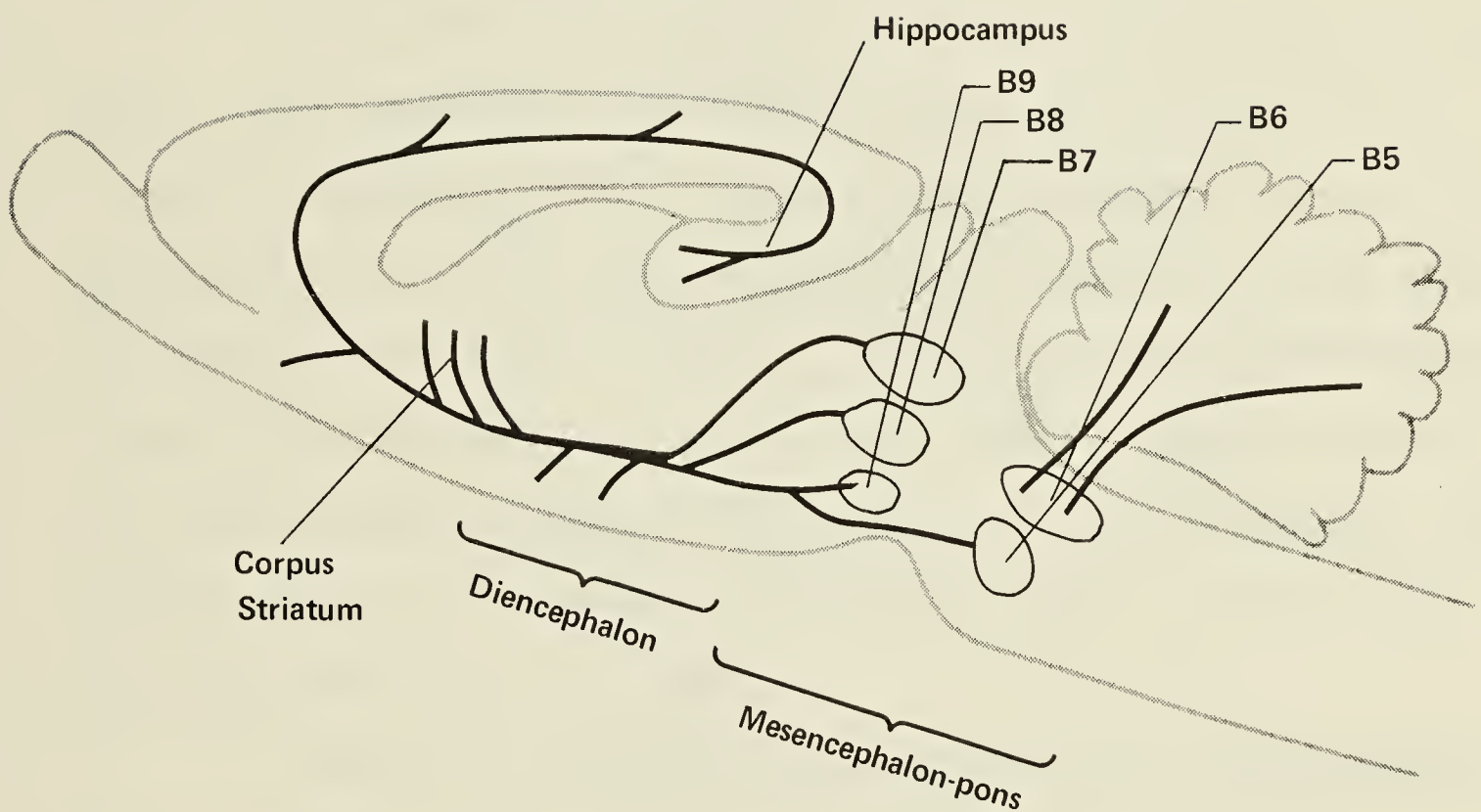


Fig. 3 Diagram of a sagittal section (after Fuxe and Jonsson, 1974) showing 5HT cell groups in rat with their projections in relation to brain areas examined in the present study.

B5 = raphe pontis, B6 = undefined, situated under the fourth ventricle, B7 = dorsal raphe nuclei, B8 = medial raphe nuclei, B9 = lateral nuclei.

The anatomical description of 5HT is by no means complete. A complete understanding of the 5HT neuronal network is yet to come.

(1.1.2.4) Summary

The main monoaminergic systems are summarized in Fig. 4.

(1.2) Metabolism of amines

(1.2.1) Dopamine and Noradrenaline

The synthesis and catabolism of DA and NA are shown in Fig. 5 and 6 . The synthesis of these amines begins with the amino acid tyrosine (Udenfriend, 1966). Tyrosine, which originates from dietary sources directly or from phenylalanine in the liver, is rapidly taken up into the brain from the plasma. Synthesis of these catecholamines occurs within the neurons (Chirigo et al., 1966). Although the brain can convert phenylalanine to tyrosine using tyrosine hydroxylase, the primary source of this amino acid for amine synthesis appears to be the plasma (Chirigo et al., 1966).

Tyrosine is first meta-hydroxylated to form dihydroxyphenylalanine (DOPA) in a reaction which is catalyzed by the enzyme tyrosine hydroxylase (Udenfriend, 1966). This enzyme is relatively specific for substrate and its regional distribution parallels that of the catecholamines. Spector et al. (1967) have shown that increasing concentrations of catecholamines in vitro diminishes the action of this enzyme. Its highest concentration is in the caudate nucleus then in descending order of concentration in the hypothalamus, the thalamus, the midbrain, the medulla oblongata, the cerebral cortex and the cerebellum (Nagatus, 1973). Its subcellular distribution suggests that it is concentrated in the nerve terminals (synaptosomes) of the brain tissue (McGeer et al., 1965) where it appears to exist

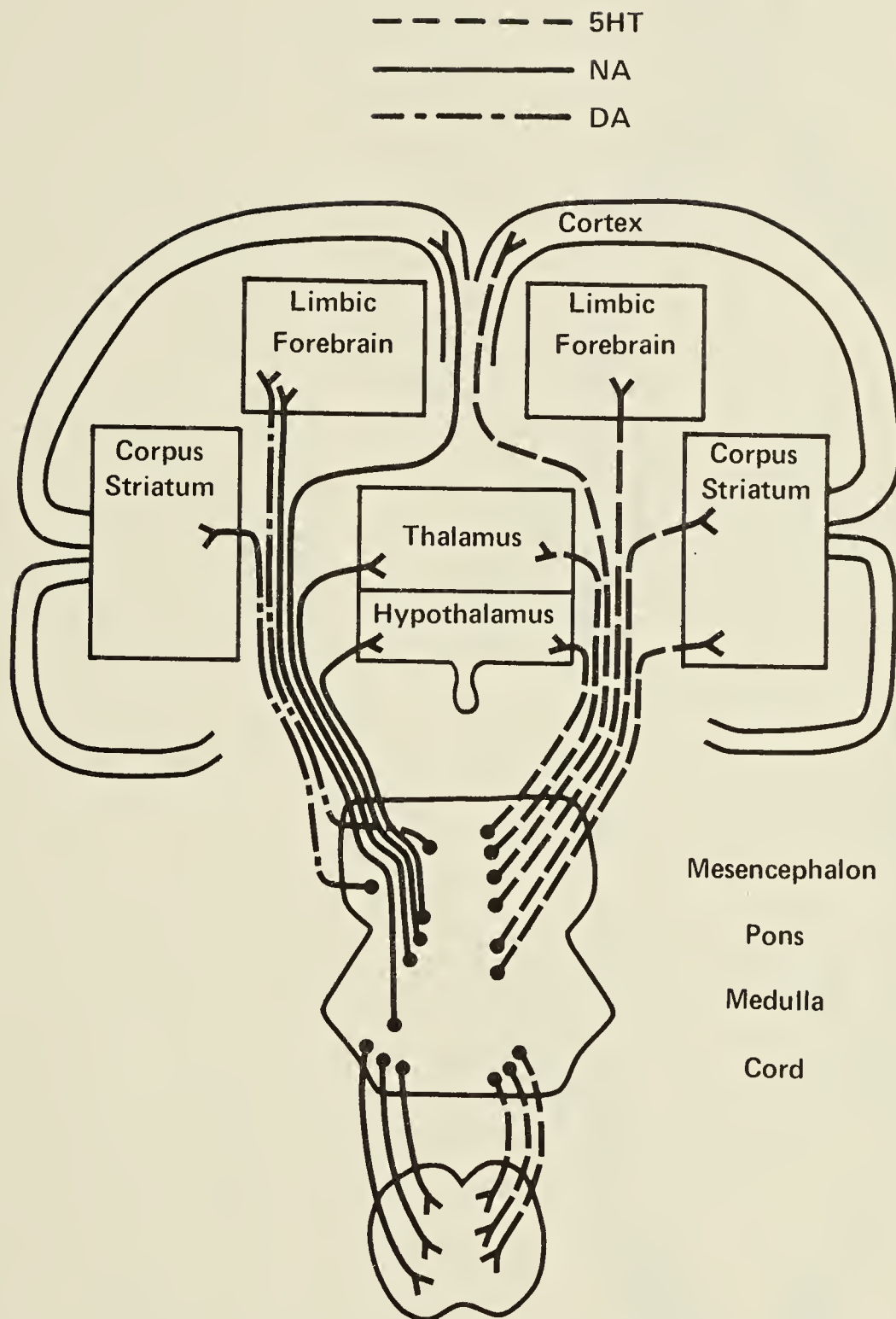


Fig. 4 Diagrammatic representation of DA, NA and 5HT fiber tracts originating from the brain stem (adapted from Andén et al., 1966).



Fig. 5 Synthesis and degradation of Dopamine

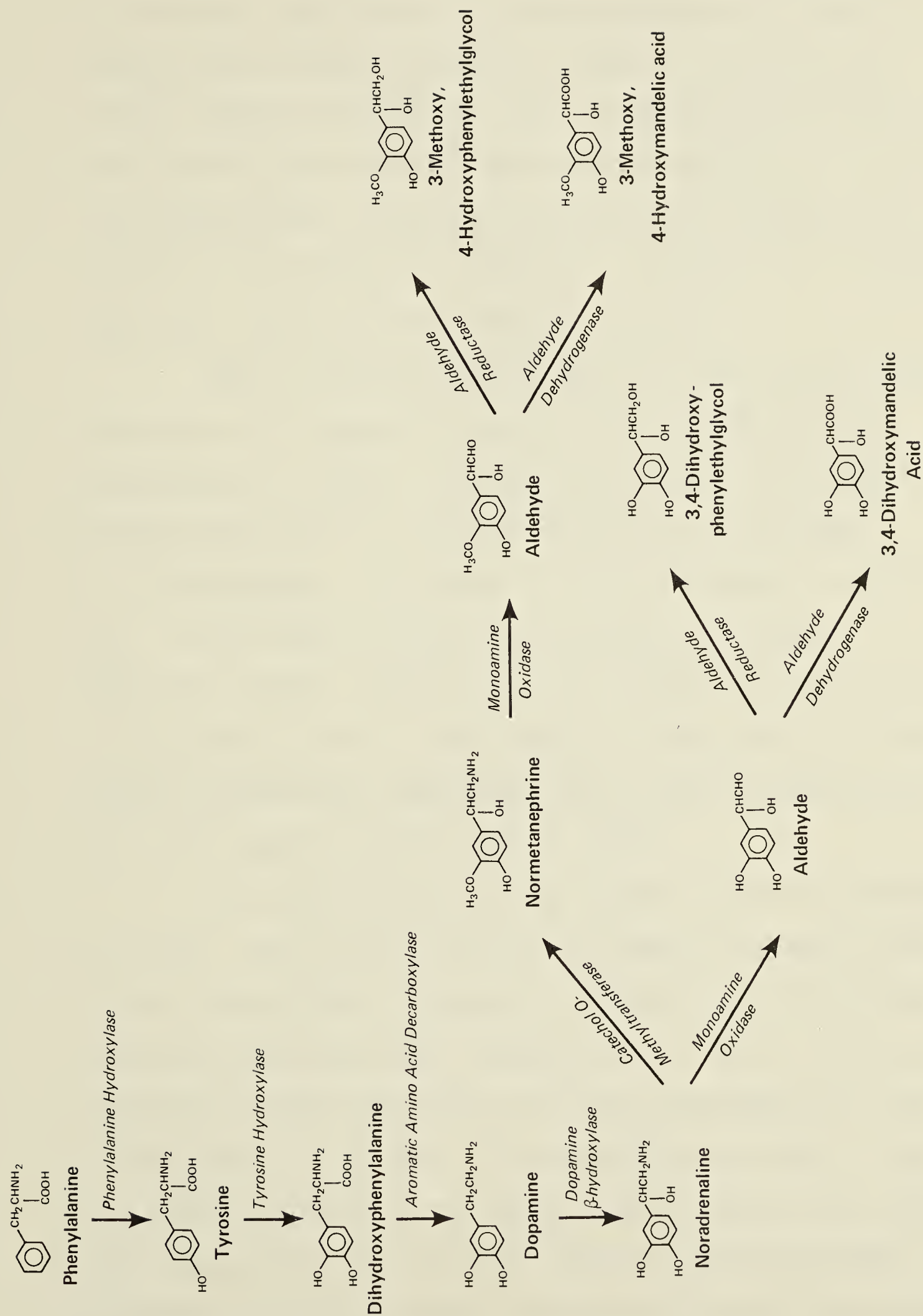


Fig. 6 Synthesis and degradation of Noradrenaline

in two forms; a particulate form, found associated with synaptic vesicles, and a soluble form which is found in the cytoplasmic fraction (Nagatus and Nagatus, 1970). Wursburger and Musacchio (1971) have shown that the enzyme is adsorbed to the particulate fractions upon homogenization, suggesting that particulate fractions may be an artifact of the technique of isolation. At present then, the actual subcellular location of this enzyme is unresolved.

DOPA is rapidly decarboxylated to DA, the reaction being catalyzed by the nonspecific aromatic amino acid decarboxylase (Lovenberg et al., 1962). The question of whether or not DOPA decarboxylase exists as a separate entity will be discussed in a later section. This enzyme is widely distributed in mammalian brain tissues where it appears to be cytoplasmic in nature (Udenfriend, 1966). DA either acts as a transmitter itself or is β -hydroxylated to form NA. The reaction is catalyzed by the enzyme dopamine β -hydroxylase (Udenfriend, 1966), which is present mainly within the synaptic vesicles (Stjarne, 1966).

The catabolism of DA and NA proceeds via two main pathways in the CNS. The principle intraneuronal metabolic pathway of both of these catecholamines is oxidative deamination to form aldehydes, catalyzed by the enzyme MAO (Blaschko et al., 1937). Two forms of the enzyme have been described in rat brain homogenates, an A and a B form (Neff et al., 1974). DA is metabolized by either a type A or type B, whereas NA is selectively metabolized by a type A MAO (Neff et al., 1974). The aldehydes formed are rapidly converted to alcohols or acids by aldehyde reductase and aldehyde dehydrogenase respectively.

The second route of catecholamine catabolism occurs through an o-methylation of the amines, the reaction being catalyzed by the enzyme catechol o-methyltransferase (COMT) (Axelrod, 1957). COMT is found in all areas of the brain primarily associated with the soluble fraction of the cell (Axelrod and Tomchick, 1958; Axelrod et al., 1959). The major sites of action of this enzyme are in the blood stream (Axelrod, 1971). The products formed by COMT catalyzed reactions are then converted to aldehydes which are in turn catabolized to alcohols and acids for excretion utilizing the same enzymes described above, MAO, aldehyde reductase and aldehyde dehydrogenase (Versteeg and Wurtman, 1976).

(1.2.2) 5-hydroxytryptamine

The synthesis and catabolism of 5HT is outlined in Fig. 7 . Synthesis in brain tissues occurs in two steps, the first a hydroxylation of tryptophan to 5-hydroxytryptophan (5HTP), the second a decarboxylation of 5HTP to the amine. The first step, which is catalyzed by the enzyme tryptophan hydroxylase, has been shown to occur readily in brain tissues (Grahame-Smith, 1964). This reaction is the rate limiting step in the synthesis of 5HT (Lovenberg et al., 1967). The distribution of this enzyme in rat brain tissues has been studied and areas of highest concentration are reported in the brain stem, hypothalamus, caudate nucleus and amygdala (Deguchi and Barchas, 1972). Two forms of the enzyme have been defined, a soluble form and a particulate form (Knapp and Mandel, 1972). The soluble form of the enzyme appears to be highly concentrated in the cell body areas of the brain and the particulate form is associated with nerve terminal areas.

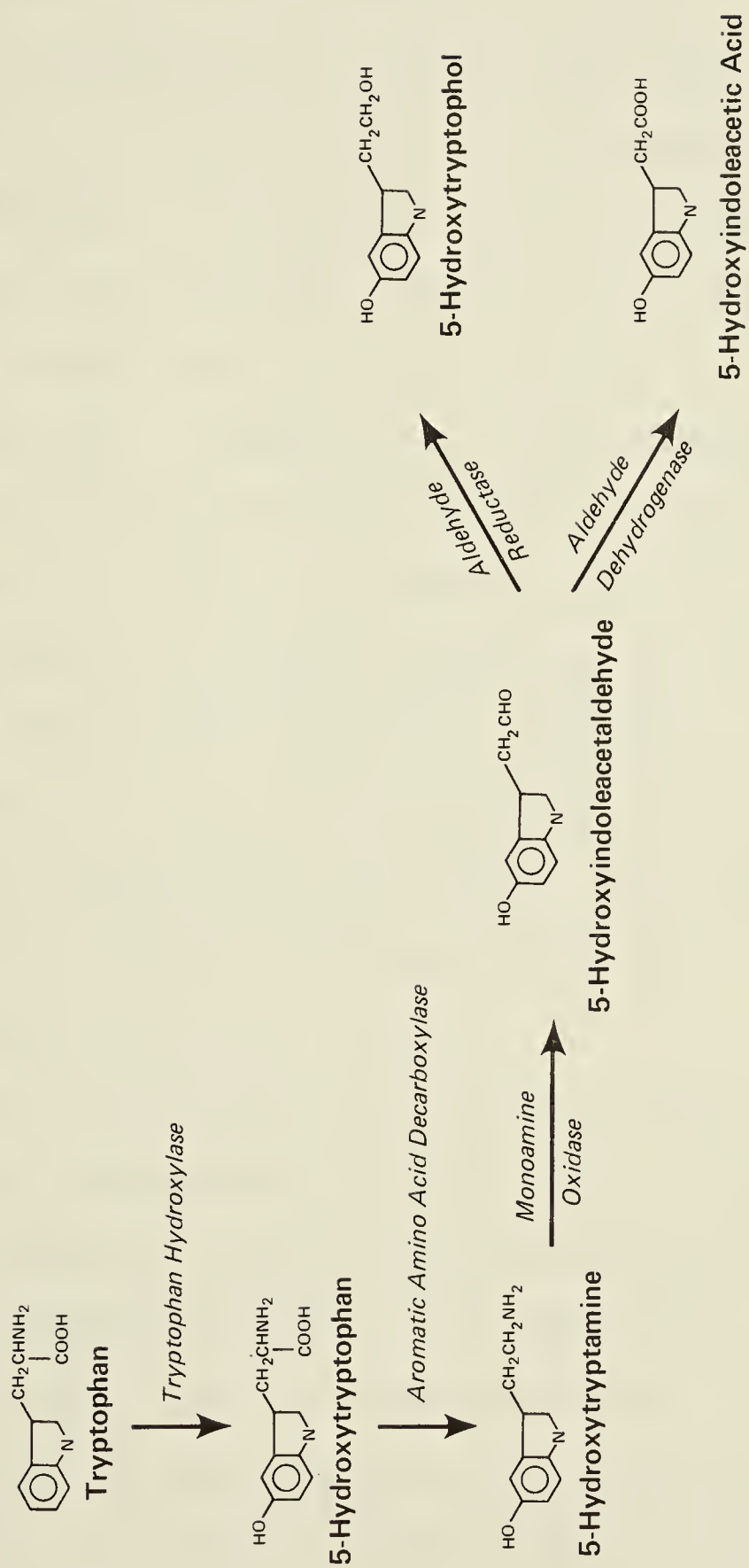


Fig. 7 Synthesis and degradation of 5-Hydroxytryptamine

Upon homogenization and subcellular fractionation of brain tissues, tryptophan hydroxylase activity is found to be highly concentrated in the synaptosomal fraction (Grahame-Smith, 1967).

The decarboxylation of 5HTP to 5HT occurs very rapidly in brain tissues in a reaction catalyzed by the enzyme aromatic amino acid decarboxylase. This enzyme is widely distributed in rat brain and has been found to be mainly associated with synaptosomal fractions. Whether or not the enzyme exists in a bound state is still debatable (Udenfriend, 1966). Lovenberg et al. (1962) presented evidence which suggested that this enzyme was non-specific in CNS tissues. However some question has arisen as to whether or not 5HTP decarboxylase and DOPA decarboxylase are in fact the same enzyme (Sims, 1974). Sims presented evidence which showed that the peripheral decarboxylase may be different from the central decarboxylase for by incubating the centrally derived enzyme with DOPA and 5HTP there appeared to be more specificity for one of the parent amino acids compared to the other suggesting two enzymes may exist.

5HT is metabolized via a series of steps similar to those involving the catecholamines DA and NA. 5HT is first converted into its aldehyde in a reaction catalyzed by monoamine oxidase (MAO) (Green and Grahame-Smith, 1975). This non-specific enzyme has been isolated from glial cells and synaptosomes where it has been associated with the outer membrane of the mitochondria (Schniatmas et al., 1967). As mentioned earlier two forms of the enzyme have been described in rat brain homogenates, an A and a B form. The type A enzyme which metabolizes NA also metabolizes 5HT (Neff et al., 1974). The aldehyde formed is rapidly either oxidized to an acid by aldehyde

dehydrogenase or reduced to an alcohol by aldehyde reductase. Both of these products are rapidly excreted by the cell.

(1.3) Synaptic physiology of biogenic amines.

The subcellular distribution of DA, NA and 5HT has been studied extensively by Whittaker (1969) and de Robertis et al. (1969). They have been shown to be present predominantly in the synaptosomal fraction. Various transport systems are utilized to accumulate amino acids and transmitter molecules into the presynaptic terminal (see Fig. 8) (Versteeg and Wurtman, 1976). Once inside the presynaptic terminal the amino acids are metabolized to the various neurotransmitters which are then stored within the synaptic vesicles. The re-uptake transport system for the amines is a principle mechanism of terminating their activity at the synapse (Iversen, 1971). The enzymes involved in the metabolism of the various transmitters are synthesized in the cell bodies and are transported to the terminals by axoplasmic flow (Whittaker, 1973).

(1.4) Normal Physiological role of DA, NA and 5HT in the CNS.

(1.4.1) Role of DA

The sensorimotor integration circuits of the striatum affect the locomotor activity of an animal and DA has been implicated as the primary neurotransmitter involved in these circuits. DA has been shown to be an inhibitory transmitter in the striatum (Barbeau, 1972). That is, by activating dopaminergic receptors, striatal outflow is reduced.

The striatum is presently believed to be involved in regulating outputs from the pallidum. Increased outflow from the striatum inhibits the motor activity controlled by the pallidum. The pallidum through the control of the striatum ensures postural

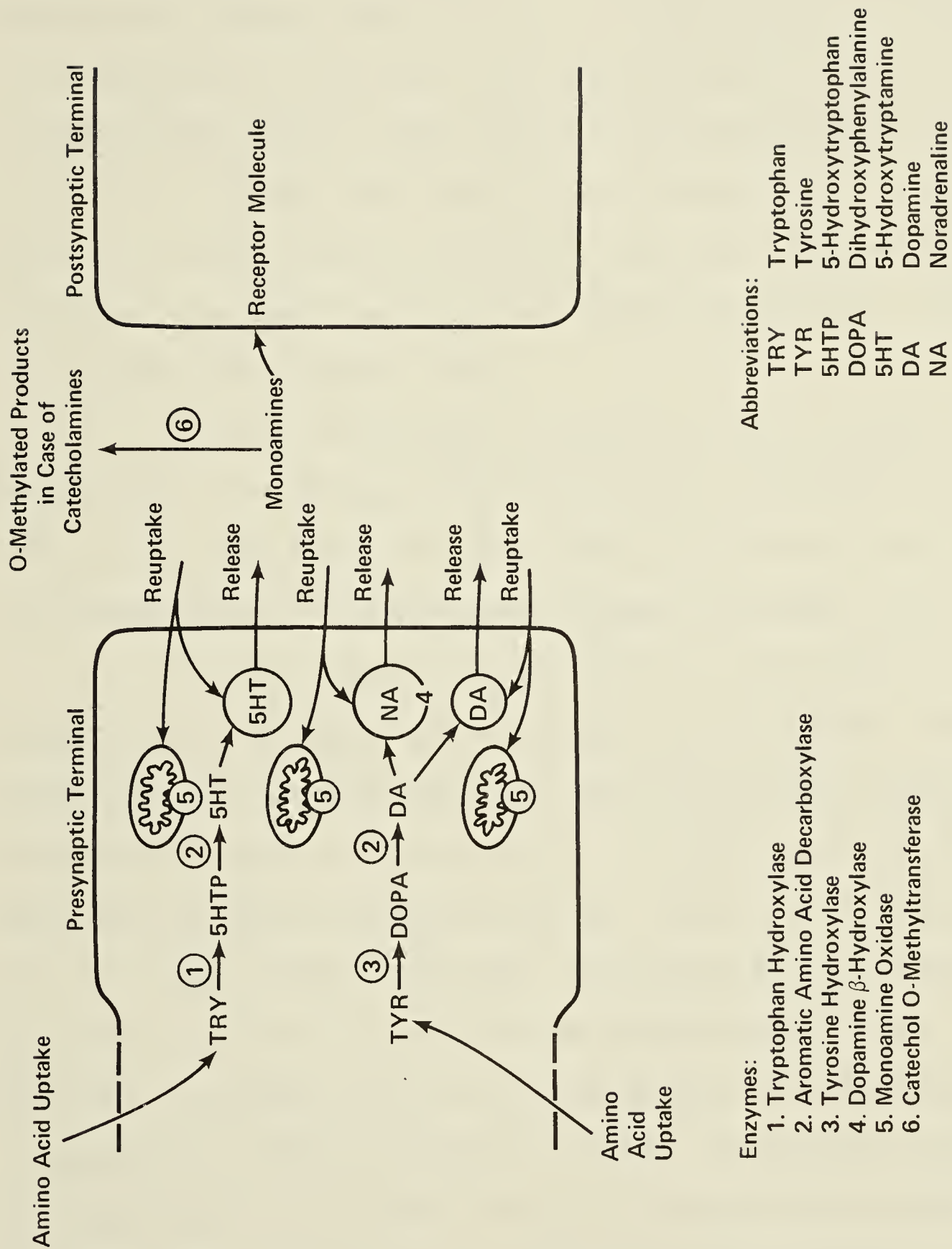


Fig. 8 Schematic diagram showing processes at the monoaminergic synaptic level in CNS.

and muscle tone to be "set" in such a way that purposeful movement can be carried out. This "set" as it is called is dependent on efficient dopaminergic systems in the striatum. DA release in the striatum leads to increased motor activity by inhibiting the output of the striatum to the pallidum which in turn releases the pallidum from its inhibitory control. On the other hand, decreased dopaminergic activity in the striatum increases outflow of striatal inhibition to the pallidum resulting in an inability to initiate movement (Ungerstedt, 1974; Bieger and Hockman, 1975; Barbeau, 1972).

This motor inactivation is similar to that in human parkinsonism. The akinesia associated with this condition is primarily affected by a reduction in DA, with tremor and rigidity probably being a secondary effect of the deficiency (Bieger and Hockman, 1975).

Unilateral stimulation or inhibition of the substantia nigra or corpus striatum results in hyperkinetic movements. These asymmetries manifest themselves in a rotational movement called contraversion, where animals rotate in a direction away from the side which has the higher concentration of DA. Thus, if DA is injected into the corpus striatum the animal rotates away from that side. If the corpus striatum is removed from one side the animal rotates towards that side. Bilateral lesioning or denervation on the other hand leads to hypokinetic, adipsic and aphagic behavior. Animals will die within 3 to 5 days after the operation if they are not maintained by tube feeding. These animals have been described as being unable to respond adequately to any sensory stimuli (Ungerstedt, 1974; Bieger and Hockman, 1975).

Present evidence suggests that DA is not the only brain component responsible for locomotor activity (Barbeau, 1972). If dopaminergic receptors are blocked the resultant motor activity is not as great as that where both DA and NA receptors are stimulated concurrently. In animals where dopamine β -hydroxylase is inhibited, motor stimulation is reduced after L-DOPA, when compared to animals who have not been treated with inhibitors (Barbeau, 1972). Further, there is a supraadditive stimulation of motor activity when NA and DA receptors are stimulated together. Thus it appears that NA is essential for the development of locomotor activity (Barbeau, 1972; Barchas, 1972; Bieger and Hockman, 1975).

Pituitary hormonal releasing factors which originate in the median eminence of the hypothalamus are released into the portal vessel system and transferred to the pituitary. It has been suggested that DA plays a central role in the regulation of synthesis and release of these factors (Mandel and Segal, 1973; Donovan, 1970; Barbeau, 1972).

Stimulation of DA receptors can lead to the development of complex stereotyped behavior patterns. Stereotyped behavior is described as a continuum of complex patterns of nonadaptive, nonpurposive compulsive behavior (Bieger and Hockman, 1975). These patterns which are species specific may include predatory behavior, oral activities such as sniffing, licking, gnawing and biting, and exaggerated motor activity. The animals appear to be unable to attend to incoming environmental stimuli. It has been proposed that dopaminergic circuits may inhibit brainstem areas which integrate incoming stimuli, and that these dopaminergic systems themselves are subject

to inhibiting control through DA synapses (Bieger and Hockman, 1975).

Evidence now exists to show that the striatum is not only an important integrating center for sensorimotor behavior but it is also important in integrating autonomic systems in the CNS. Autonomic systems are modulated through inhibitory control upon the pallidum, hypothalamus and brainstem (Barbeau, 1972). The present view is that DA acting as a modulator in the striatum, plays an indirect role as a feedback component of the autonomic system. In so doing the striatum is believed to be important in maintaining the homeostasis of this system.

The striatum receives neuronal and humoral feedback on the status of the sympathetic and parasympathetic systems of the body. This feedback comes not only from the peripheral receptors but also from centers in the hypothalamus and brainstem. As long as the sympathetic and parasympathetic systems remain in balance, the striatum remains quiescent. Once an imbalance arises the striatum responds with an increased turnover of DA which results in modifications to other systems in the CNS.

Until recently, acetylcholine (ACh) was believed to be the only transmitter in the CNS active in the parasympathetic nervous system. Present evidence however shows that 5HT may also be extremely important in the mediation of activity along this system in the CNS (Barbeau, 1972). 5HT appears to be involved with conduction of outflow along longer pathways whereas ACh plays a role in shorter integrating circuits. The parasympathetic system can be visualized then

as a series of long serotonergic and short cholinergic pathways where one modulates the other.

DA appears to act as a regulator of cholinergic activity. It may exert this control through inhibition of ACh release at presynaptic sites. To date, there has been no evidence to suggest that axoaxonal synaptic linkages exist between ACh and DA. DA may also act upon 5HT through a variety of mechanisms including competition of precursor amino acid uptake, competition for enzyme systems important in the synthesis of the two transmitters, and a competition for reuptake and displacement at synaptic sites (Barbeau, 1972). Displacement refers to the uptake of DA into serotonergic or noradrenergic terminals and a displacement of the native amine from storage sites by DA. DA is then released as a false transmitter (Barbeau, 1972; Bieger and Hockman, 1975). Thus it appears that DA could control parasympathetic outflow through either inhibition of ACh release or interference with 5HT terminal activity. Likewise, DA could interfere with the normal metabolism and release of 5HT and NA.

(1.4.2) Role of NA

It is commonly believed that the normal physiological effects of NA on behavior are an activation of arousal systems and emotion. Increased NA concentrations in animals results in hyperactivity; decreased NA in brain results in decreased activity (Mandel and Segal, 1973).

The reputed central excitation of NA has been viewed by others as mediated by the peripheral effects of this amine. For example, the arousal noted after the administration of NA is due to a rise in blood

pressure (Dewhurst, 1968; Marley and Stephenson, 1972). Earlier work had shown that NA produced CNS depression in the chick (Dewhurst and Marley, 1965). When NA was infused into the midbrain reticular formation of rats the observed behavioral pattern was a reduced locomotor activity although drowsiness was not noted (Marley and Stephenson, 1972). Other work cited by these authors has shown that if NA is administered intracisternally or intraventricularly the observed behavior pattern in dogs and cats is a sleepy drowsy state. Other authors have cited evidence for an anesthetic-like state with notable stupor and sedation which were probably the result of a toxic infusion dosage of NA (Mandel and Segal, 1973). An anesthetic-like action was not noted by Marley and Stephenson (1972) because animals responded to painful stimuli.

NA has been implicated as the hypothalamic modulator of eating and drinking behavior. NA injected into the dorsomedial or lateral hypothalamus induces feeding in both satiated and starved rats. Adrenergic antagonists block this behavior and antagonize NA injections into this area (Berger, Wise and Stein, 1971). Stimulation of the medial forebrain bundle which contains noradrenergic fibres projecting into the hypothalamic region produces eating. Lateral hypothalamic damage results in a reduction of feeding behavior (Berger, Wise and Stein, 1971). When NA is infused into the anterior hypothalamus or preoptic area, the response of the animal depends on the state of deprivation. In animals who were food deprived, NA injection resulted in increased drinking and decreased eating whereas in thirsty animals NA injections produced increased eating and decreased water intake

(Hutchenson and Renfrew, 1967).

NA and 5HT have been implicated as control modulators of many systems in the brain. Since it is difficult to talk of one without referring to the other, further consideration of NA will be discussed under the next heading.

(1.4.3) Role of 5HT

Evidence for a serotonergic involvement in sleep comes from three different observations. p-Chlorophenylalanine (pCPA), a reputed selective tryptophan hydroxylase inhibitor (Koe and Weissman, 1966) when given to cats results in a decrease in both slow wave sleep and paradoxical sleep after 18 - 24 hours, with complete insomnia apparent after 30 - 40 hours. This correlates with the progressive decrease in CNS 5HT levels after the pCPA treatment (Koe and Weissman, 1966). When cats are treated with pCPA, normal patterns of sleep appear again after 200 hours. If these animals are given an injection of 5-hydroxytryptophan (5HTP) however, normal patterns of sleep arise within 6 - 8 hours of the 5HTP injection.

Destruction of 80 - 98% of the raphe system in the cat results in permanent behavioral arousal lasting 3 - 4 days. Following this period, slow wave sleep is reduced to 10 - 15% of sleep time (normally slow wave sleep is 80%). Paradoxical sleep is completely blocked (Jouvet, 1974). It appears then that the raphe system may be the anatomical location of centers responsible for the induction of sleep.

5HT appears to be responsible for triggering the onset of sleep. How 5HT accomplishes this onset is at present obscure.

Possibly, 5HT release could act directly through the reticular activating system to inhibit cortical arousal, or it could act on catecholamine containing neurons within the mesencephalic reticular formation inhibiting their activity.

That NA is involved in sleep regulation is supported by two pieces of experimental evidence. First, increases in cerebral catecholamines after L-DOPA induce a long-lasting arousal in the cat, and second if the synthesis of these amines is inhibited by α -methyl-p-tyrosine normal waking behavior is decreased (Jouvet, 1974). Because destruction of the substantia nigra does not have any effect on cortical arousal, NA is considered to be responsible for these effects. Increases of NA levels have also been correlated with the onset of paradoxical sleep (Jouvet, 1968, 1974; Barchas, 1972; Green and Grahame-Smith, 1975).

The L-DOPA evidence for involvement of NA in cortical arousal can be questioned on two counts, however. When L-DOPA is administered peripherally a great majority of it is decarboxylated there. In so doing, peripheral NA levels would increase and observed increased cortical arousal could be then due to the effects this amine has on blood pressure (Dewhurst and Marley, 1965). Secondly the majority of L-DOPA reaching the CNS is converted to DA (Barbeau, 1972). Increased DA acts centrally in the corpus striatum resulting in marked locomotor excitability (see earlier). The α -methyl-p-tyrosine evidence for involvement of NA in arousal can also be criticized. Administration of α -methyl-p-tyrosine decreases the levels of NA and DA in the CNS. Reduced levels of DA have been shown to result in a

motor retardation which could appear as reduced arousal.

The involvement of 5HT in sleep appears to be unquestionable.

Whether or not NA acts centrally by triggering arousal is at present a debated issue. As with other physiological processes there are marked species differences when discussing the effects of particular compounds in the regulation of this process.

NA and 5HT also play a predominant role in thermoregulation (Green and Grahame-Smith, 1975; Marley and Stephenson, 1972). Anatomically, this regulation appears to occur in the anterior hypothalamus. The role of each of the amines is dependent on species, dosage and route of administration. In general, it appears that NA decreases body temperature while 5HT increases it. The actual mechanism of this regulatory process is still a debated issue. Whether the amines are acting directly on effectors in the hypothalamus, on blood supplying the hypothalamus or indirectly through release of amines such as ACh in other areas is as yet unresolved.

Evidence for the involvement of 5HT in the mediation of pain has been shown (Green and Grahame-Smith, 1975). When ascending serotonergic pathways are lesioned, there is a significant increase in sensitivity to electrical foot shock. 5HTP will reverse this increased sensitivity. pCPA will produce a marked increase in sensitivity to pain. Others have shown that foot shock results in increased NA, DA and 5HT turnover in rats (Barchas, 1972). The brain area of investigation is critical in assessing the extent to which each amine is effective. Evidence has also been presented to suggest that the synthesis of NA is increased after foot shock (Barchas, 1972). It is evident

then that a more thorough investigation of the action of 5HT, NA and DA with respect to pain perception is essential (Chase and Murphy, 1973; Green and Grahame-Smith, 1975).

5HT has been implicated in hypersexual behavior in that if pCPA is given to male rats or rabbits the animals show a marked increase in sexual drive (Gessa and Tagliamonte, 1974). This increased sexual activity after 5HT depletion is species specific; it is observed in male rats and rabbits, is questionable in cats and has been shown not to occur in monkeys. This increased sexual activity observed in rats can be abolished by injections of 5HTP and can be enhanced by monoamine oxidase inhibitors (MAOI) (Green and Grahame-Smith, 1975). Because this activity can be increased or enhanced by MAOI, it has been suggested that 5HT inhibits sexual activity while the catecholamines enhance sexual activity because MAOI would increase the level of the catecholamines (Chase and Murphy, 1973; Green and Grahame-Smith, 1975). L-DOPA administration coupled with a peripheral decarboxylase inhibitor has been shown to increase sexual activity in sexually sluggish rats. Likewise, administration of apomorphine potentiates sexual activity in these rats (Gessa and Tagliamonte, 1974). Both of these pieces of evidence suggest that DA may increase sexual activity. Clearly then, an increase in sexual activity is not due solely to a reduction of central 5HT levels. Because MAOI increases the levels of all amines that are metabolized by monoamine oxidase and especially the trace amines (Boulton, 1976) other transmitters may also be involved.

Stein and Wise (1976) have suggested that there is a noradrenergic facilitatory reward system and a serotonergic

suppressant punishment system in the brain. These authors have studied self-stimulation of the medial forebrain bundle (MFB) in rats. They found that drugs which enhance self-stimulation release catecholamines while drugs which inhibit self-stimulation deplete tissues of their catecholamines. Stimulation of the MFB which contains important ascending noradrenergic fibre tracts results in release of NA and its metabolites from areas in the hypothalamus and amygdala (Stein and Wise, 1967).

5HT on the other hand is associated with reduced self-stimulation (Stein and Wise, 1974). Likewise, when the synthesis of 5HT is suppressed by compounds such as pCPA, self-stimulation is increased. When methysergide, a 5HT receptor blocker, was administered to rats punishment behavior was antagonized. Punishment behavior was also antagonized by administering NA. On the other hand this behavior was facilitated by pentolamine (α -adrenergic receptor blocker) and α -methyltryptamine a potent 5HT agonist (Stein and Wise, 1974).

Ungerstedt (1974) has recently presented evidence to suggest that self-stimulation may be elicited from centers known to contain large numbers of DA neurons. Thus it appears that DA neurons are necessary for self-stimulation. What role they play is not yet known.

(1.5) Role of cerebral amines in the pathology of mood

(1.5.1) The Biogenic Amine Theory of Affective Psychoses

One basis for a biogenic amine theory of the affective psychoses came from pharmacological evidence. Drugs that are effective clinically in the management of this disease have been reputed to alter the concentration of these amines at the synapse. Those compounds which are effective in the treatment of depression have been found to increase the levels of the biogenic amines at the synapse. Those compounds which are effective in the treatment of mania have been shown to decrease the concentration of these amines at the synapse. Thus, the biogenic amine theory states that there is an over-abundance of amine available for synaptic transmission in mania and that there is a reduction in the amount of transmitter in depression.

One of the first hypotheses to emanate from the pharmacological evidence was the catecholamine theory (Schildkraut, 1965; Bunney and Davis, 1965) which proposes that depression is due to a deficiency of the catecholamines, particularly NA, at the noradrenergic receptor sites in the brain. Mania on the other hand is due to an excess of these catecholamines at CNS receptors.

Evidence presented by Schildkraut (1965) for the catecholamine theory includes:

1. Monoamine oxidase inhibitors which are effective anti-depressants raise the levels of brain NA
2. Amphetamine which is active in the treatment of depression is believed to act by releasing NA and effectively blocking neuronal reuptake

3. Imipramine which is also effective in the treatment of depression has been found to block reuptake of NA into the presynaptic terminals
4. Reserpine, which results in depressive like symptoms in some patients, has been shown to deplete NA from central storage sites.
5. L-DOPA, the precursor of NA, has been found to result in mood elevation.

Criticisms of this evidence as reviewed by Dewhurst (1969) and Mendels and Frazer (1974) will be presented here. Monoamine oxidase inhibitors protect all amines which are actively deaminated by this enzyme (Dewhurst, 1969). Amphetamine has a direct action on receptors responsible for arousal (Dewhurst, 1969). Imipramine also increases the levels of other amines in the CNS and reserpine depletes other amines as well as NA (Dewhurst, 1969). In controlled clinical studies reserpine resulted in no depressive symptoms. The resultant behavior was described as a psychomotor retardation, thought to be a consequence of the reduction of DA centrally (Mendels and Frazer, 1974). L-DOPA studies have been contradictory with some workers being unable to replicate the increased arousal associated with L-DOPA treatment (Dewhurst, 1969). Clearly then, the evidence presented originally can be interpreted in various ways. What is necessary for a clearer understanding of the role of NA in mood changes is a compound which could be more selective in its action on NA. One such compound, α -methyl-p-tyrosine has been used to selectively block the production of these catecholamines. This compound though, depletes

both peripheral and central levels of the catecholamines. Since changes in peripheral levels of these amines can effect behavior, behavioral changes noted after this drug must be related with caution to changes in mood. In general, clinical trials with this compound have not produced any appreciable changes in mood. The primary complaint after treatment was that of fatigue, with some reports of anxiety reactions. When α -methyl-p-tyrosine was administered to psychiatric patients, only two of seven manic patients treated showed improvement associated with the drug treatment while one manic patient unexplainably deteriorated. In all three depressed patients deterioration was characterized by psychomotor retardation, possibly due to a decrease in DA (Mendels and Frazer, 1974).

A second hypothesis originally proposed by Lapin and Oxenkrug (1969) implicated 5HT in the etiology of the affective psychosis. These authors suggest that decreases of brain 5HT lead to behavior characteristic of depression, while mania results from increased brain levels of 5HT. Evidence presented by Lapin and Oxenkrug (1969) for the involvement of 5HT in the etiology of diseases of affect includes:

1. Monoamine oxidase inhibitors increase the levels of amines including 5HT in the brain. Enhancement of the antidepressant effects of monoamine oxidase inhibitors by injections of tryptophan supports involvement of 5HT
2. Imipramine-like compounds act on 5HT in much the same fashion as they act on NA, by increasing its concentration at the synapse

3. Tryptophan a precursor of 5HT has been shown to produce antidepressant effects.

Like the evidence presented for the catecholamines, the evidence in support of the 5HT hypothesis has been criticized (Dewhurst, 1969; Mendels and Frazer, 1974). The evidence based on the use of monoamine oxidase inhibitors and imipramine has been criticized because of their non-specific action (Dewhurst, 1969). 5-Hydroxy-tryptophan treatment in controlled trials has given conflicting results (Kline, Sacks and Simpson, 1964). In clinical trials, pCPA has not resulted in behavior indicative of depression. Side effects including tiredness and anxiety were reported but symptoms were non-specific and self-limiting (Mendels and Frazer, 1974).

A third hypothesis has been presented to link amines to affective disorders (Dewhurst, 1968). This hypothesis started with the argument that if monoamine oxidase inhibitors can elevate mood in man then they presumably do so by altering one or other of the substrates of monoamine oxidase. As a result of studies in man it was shown that tryptamine was the most affected by such drugs and catecholamines least with 5HT intermediate. A further detailed examination of these and other amines in animals under rigid environmental and social control led to the separation of two main groups of amines.

Type A amines were soluble in fat and water and were unhydroxylated aromatic ethylamines such as tryptamine and phenylethylamine. Specific cerebral receptors were defined for this group by the use of a specific antagonist methysergide. Physiologically these amines produced all the phenomena of the alert state with

exploratory and aggressive activity and accompanying increase in EMG activity as well as a desynchronized EEG characteristic of the alert state. This group includes substances such as amphetamine but the most potent natural agonists are tryptamine and phenylethylamine.

On the other hand Type C amines are all water soluble but sparingly soluble in fat and are characteristically hydroxylated aromatic ethylamines such as noradrenaline and adrenaline. Physiologically, when introduced into the CNS, these amines diminish locomotor and EMG activity, produce behavioral sleep and an EEG typical of this state. Again receptors have been defined in the CNS for these amines and they are similar to the peripheral α receptor for catecholamines.

The Type A amines and receptors as defined in the above animal experiments can be linked to mood in man without anthropomorphism because it has been observed that: (1) Type A amines such as amphetamine produce euphoria in man, (2) tryptophan the precursor of tryptamine has also a euphoric action in man and (3) methysergide a specific antagonist of the Type A receptor blocks such mood elevation.

Thus it is deduced that naturally occurring Type A amines such as tryptamine and phenylethylamine are essential for normal mood in man (in appropriate psycho-social circumstances) and by extending this view into pathological states it postulates that some forms of depressive illness are due to deficiencies in Type A amines or desensitization of Type A receptors while some if not all forms of mania result from an excess of Type A amines or supersensitivity of Type A receptors.

In both cases psycho-social factors are emphasized as well as amine change.

Evidence for this hypothesis of affective disorders includes:

1. Monoamine oxidase inhibitors increase levels of all amines including the excitant amines. Interestingly though, the levels of tryptamine in mouse brain increase 96 fold after tranylcypromine and 35 fold after pargyline (Tabakoff et al., 1977). In the rat the levels of tryptamine increase 218 fold after pargyline while in the rabbit 311 fold increases after pargyline have been reported (Boulton, 1976). Phenylethylamine increases 103 fold in the rat brain after pargyline and 27 fold in the rabbit (Boulton, 1976). These increases are dramatic when compared to increases in the classical neurotransmitters after nialamide. 5HT increases 4 fold, NA increases 1.6 fold and DA increases 1.8 fold in mouse brain after nialamide treatment (Modigh and Svensson, 1972).
2. Tryptophan is the precursor of tryptamine as well as 5HT. Recently, Tabakoff et al. (1977) have shown that in mouse brain the levels of tryptamine increase 172 fold when tryptophan is administered in the presence of a monoamine oxidase inhibitor.
3. Other supportive evidence comes from the action of a specific tryptaminergic receptor blocker, methysergide (Dewhurst and Marley, 1965) in the treatment of mania. This drug has been found by some workers to be effective in the treatment of this disease state (Dewhurst, 1968; Haskovec and

and Soucek, 1968; Haskovec and Soucek, 1969; Verster, 1963; Serry, 1969). Others have found that the drug is ineffective (Coppen et al. 1969; Fieve et al., 1969; Grof and Foley, 1971; McCabe et al., 1970; McNamee et al., 1972). We have found that the absorption of methysergide after oral administration is extremely erratic and unpredictable in different individuals (Dewhurst and McKim in preparation). Therefore, it is essential to monitor blood levels of this drug in evaluating its clinical efficacy.

DA has not been thought to play a direct role in the etiology of the affective disorders. It may play an indirect role through alterations in the motor activity. Elevated levels of DA could produce a motor excitability which may play a role in mania while a deficiency of DA in the CNS may contribute to a psychomotor retardation (Barbeau, 1972).

(1.6) Pharmacological and biochemical effects of p-chloroamphetamine (pCA) on amines.

pCA, which is structurally similar to Type A amines has been shown to be effective in the treatment of depression (Van Praag et al., 1971). Because of the clinical efficacy of this compound a description of its action in the CNS has practical as well as theoretical implications and is therefore the subject of the following review and experimental work.

Thirteen years have passed since pCA was first shown to selectively reduce the levels of 5HT and 5HIAA in rat and guinea pig brain tissue (Pletscher et al., 1964). The drug was thought to

exhibit species differences for in rabbits and mice the drug has little or no effect on the levels of these two indoles. The above authors also found that in rats and guinea pigs a single injection of the drug (25 mg/kg) resulted in a maximal depletion of 5HT and 5HIAA 16 hours after treatment.

Fuller et al. (1965) studied the effects of chlorinated analogues of phenylethylamine in rats. Their results show an α -methyl group such as is found in amphetamine, was essential for effects to be exhibited on cerebral amine levels. Unchlorinated amphetamines had no effects on 5HT levels, whereas chlorine substitution in the 4 or para position was most potent followed by substitution in the 3 or meta position. These compounds were shown to have little or no effect on the cerebral levels of NA.

Fuller and Hines (1966; 1967) reported that amphetamine disappeared more rapidly than pCA from both mouse brain and rat brain. It had previously been found that pCA had a depleting effect on cerebral 5HT whereas amphetamine did not (Fuller et al., 1965). The particulate-supernatant distribution of these two drugs was studied and it was found that amphetamine was more closely associated with the supernatant fraction and pCA was found bound in the particulate fraction. This suggested a possible mechanism whereby these two drugs existed for different periods of time in brain tissue and might explain the differences in action of amphetamine and pCA on cerebral 5HT. It did not explain the differences of action of pCA between mouse and rat brain since in both the drug was associated with the particulate fraction.

An alternative explanation could be related to the metabolism of these two compounds. In rats, amphetamines are normally para-hydroxylated for excretion (Dring et al., 1970). A chlorine group in the para position of the phenolic ring of the drug could prevent this normal metabolic process. In mice, amphetamines are normally deaminated for excretion (Dring et al., 1970). Since Fuller and Hines (1967) found that pCA was cleared at a similar rate from these two species this explanation could not hold since it would have been expected that pCA would have a greater half life in rat brain than in mouse brain.

In direct disagreement with this work Miller et al. (1971) have shown that there is a marked difference in the half life of pCA (10 mg/kg) in mice (4hours) and rats (10 hours). Sixteen hours after a single injection of pCA the drug was not detectable in mouse brain but was still measurable in rat brain. Amphetamine on the other hand had a similar half life of 1.5 hours in both mice and rats. The level of 5HT in mouse brain declined to 60% of control at 6 hours after a pCA injection with the level returning to normal at 16 hours. In rats the levels of 5HT had declined to 25% of control at 6 hours and remained at 30% of control 16 hours after an injection of pCA. Thus it appears that the metabolism of this drug in these two species contributes to the differences in its effect on cerebral 5HT levels.

Later Fuller et al. (1972) studied the effects of amphetamine and o-chloroamphetamine, neither of which had any significant effect on levels of brain 5HT. m-Chloroamphetamine resulted in a slight but significant reduction in 5HT and pCA caused a marked and highly significant reduction in 5HT levels 6 hours after drug treatment.

Amphetamine, o-chloroamphetamine and m-chloroamphetamine were all metabolized within 6 hours of drug administration. After desmethylimipramine treatment which is supposed to inhibit para-hydroxylation, amphetamine and o-chloroamphetamine still had no effect on brain 5HT levels whereas m-chloroamphetamine produced a reduction of 5HT similar to that produced by pCA. Treatment with desmethylimipramine had no effect on pCA effects. After desmethylimipramine treatment amphetamine was still present in substantial amounts as were the chlorinated analogues. Amphetamine was found associated mainly with the supernatant fraction; o-chloroamphetamine was equally distributed between the supernatant and particulate fraction; and m- and p-chloroamphetamine were found in the particulate fraction.

Although original reports on the effects of pCA indicated that there was no effect on the catecholamines, more recent work, especially regional studies, suggests that there might be some alteration in the levels of these amines. Miller et al. (1970) reported that there was an initial effect on NA levels after an injection of 3.5 mg/kg of pCA. NA concentrations were increased in all areas studied including whole brain although no significant changes were found in the hypothalamus or midbrain. This increase lasted for only 2 hours following which levels returned to normal. 5HT and 5HIAA levels slowly decreased and after 2 hours all areas were significantly reduced except in the cerebellum where a significant reduction was recorded only after 4 hours. Although these authors suggested this initial action on NA was probably due to MAO inhibition it could result also from enhanced release.

Strada et al. (1970) have described the effects of pCA on an intraventricularly injected load of tritium-labelled (^3H) NA. They found that pCA increased the concentration of ^3H -normetanephrine and that little if any of the tritium could be found associated with the deaminated products. ^3H -NA which is introduced intraventricularly has been shown to be taken up into NA storage vesicles and is selectively released into the synaptic cleft suggesting that amines which are deactivated through uptake processes are stored close to the synaptic membrane (Iversen, 1971). The fact that the majority of the ^3H was associated with the normetanephrine product suggests either that reuptake is blocked, shunting the metabolism of the amine into the COMT pathway or that MAO is being inhibited by the drug. Since the proportion of the label associated with the deaminated o-methylated products was reduced also, the inhibition of MAO seems more likely. Using a push-pull cannula, Strada and Sulser (1971) have reported that pCA causes a 10-fold increase in release of ^3H -NA from the hypothalamus. Thus both an MAO inhibiting and a noradrenergic releasing effect seem to result from pCA treatment.

Costa et al. (1971) using very small doses of drug (0.1 - 1.5 $\mu\text{g/kg}$) in rats reported that pCA treatment results in a marked decrease of 5HT (50% of control) in the telencephalon and diencephalon after 24 hours. NA levels in the brainstem, telencephalon and diencephalon were not reduced but their results suggested a decreased turnover of DA in the corpus striatum after pCA.

Morgan et al. (1972) studied the effects of various amphetamine analogues on brain amines. They found that NA was slightly reduced in the telencephalon when measurements were made 4

hours after treatment with pCA (5 mg/kg). 5HT levels fell significantly in this area to 20 - 35% of control depending on whether the laevo or dextro isomer of pCA was used, the latter being more effective. In the hypothalamus and brainstem the levels of 5HT fell to 60 - 65% of control. When the accumulation of neurotransmitters was studied in brain slices, pCA was recorded to markedly inhibit the accumulation of NA into brainstem and corpus striatum tissues and the accumulation of 5HT into the brainstem was minimally but significantly reduced. These authors also reported that MAO activity in brain slices was inhibited 86% by pCA. There was no evidence of an inhibiting effect on tryptophan hydroxylase in vitro by this drug. These workers have reported that pCA has no effect on the concentration of tryptophan in the telencephalon 4 hours after drug treatment at which time 5HT was greatly reduced. This suggests that pCA is not interfering with the availability of tryptophan for 5HT synthesis in terminal areas. The evidence for a direct inhibition of MAO supports earlier work of Fuller and Hines (1970) who had reported that after pCA treatment (20 mg/kg) the amount of 5HIAA was markedly reduced but 5HTP and 5HT were similar to controls after a ^3H -5HTP load.

Costa and Revuelta (1972) measured the effects of an injection of pCA (5 mg/kg) on the metabolism of ^{14}C -tryptophan administered 4 hours after pCA treatment. The decline with time of the specific activity measured as tryptophan in the telencephalon and diencephalon in control and drug treated animals was similar. The accumulation of radioactive-labelled 5HT, however, was slower in the drug treated than in control animals. They also reported that the turnover of

5HT was slower in animals receiving pCA than in control animals especially in the telencephalon and diencephalon.

Leonard (1976) has recently described the changes occurring in NA, DA and 5HT in specific areas of the rat brain after pCA. Animals received 20 mg/kg pCA and were sacrificed 0.5, 1, 2, 3, 4 and 24 hours after drug treatment. For area analysis studies, animals were sacrificed at 2 hours and cerebral cortex, striatum, midbrain (hypothalamus, thalamus, hippocampus, tegmentum and colliculus), brain stem (pons and medulla), and cerebellum were all removed. After a single injection of pCA there was a rapid and marked reduction of 5HT and 5HIAA in rat brain tissues. At 3 hours after injection the level of 5HT was lower than at other times and the concentration of tryptophan was increasing at this time. Tyrosine and dopamine concentrations increased over the first 4 hours while NA concentrations remained unchanged. At 24 hours NA and tyrosine remained the same as controls. DA levels were not measured at this time. Two hours after administration of pCA the midbrain region showed the greatest reduction in 5HT; only a slight nonsignificant reduction occurred in the cortex, striatum and cerebellum. DA levels increased in most areas but significant changes occurred only in the brainstem. The concentrations of NA and tyrosine were unaffected at this time. pCA increased the conversion of radioactive-labelled ^3H -tyrosine to DA and radioactive-labelled ^3H -tryptophan to 5HT but did not increase the conversion of DA to NA. These results then support previous evidence that there is some effect on the metabolism and release of NA and DA.

This evidence suggests that the action of this drug is found in

areas which have high concentrations of axonal terminals. Wong et al. (1972) described the subcellular localization of pCA in rat brain tissues. Only 14% of the pCA was found in the high speed supernatant whereas 50% of it was particulate bound. Upon further fractionation pCA was found to be concentrated in the synaptosomal fraction. These authors suggested that pCA may act by competing for 5HT uptake into the synaptosomes. They found that pCA inhibited the high affinity uptake of 5HT but that low affinity uptake was only modestly affected (Wong et al., 1973), thus supporting their original contention that pCA may be competing with 5HT for the amine pump. They also found that pCA released 5HT from synaptosomal fractions. The uptake of NA was also inhibited by pCA.

Although earlier reports suggested that tryptophan hydroxylase was not affected by pCA (Pletscher et al., 1964; Fuller et al., 1965) the observation that 5HT and 5HIAA levels were both decreased in cerebral tissues (Fuller et al., 1965) suggested a metabolic action for the drug. Sanders-Bush and Sulser (1970) found that if ^{14}C -5HTP was introduced intraventricularly into pCA (5mg/kg) treated rats ^{14}C -5HT was still synthesized. When the drug was administered 16 hours prior to an intraventricular injection of ^{14}C -5HTP and the levels of metabolites were measured 6 hours later, both ^{14}C -5HT and ^{14}C -5HIAA were found in these animals. When animals were pretreated with pCA for 21 hours and pargyline (a MAO inhibitor) was administered, the accumulation of 5HT after 4 hours was markedly lower (25%) than in animals treated with pargyline alone, suggesting that less 5HT was formed in the 4 hour time period in the pCA treated

rats or that clearance of 5HT was enhanced. In animals treated with pCA 48 and 24 hours prior to a probenecid treatment (a drug which slows the clearance of 5HIAA from the CNS) there was no accumulation of 5HIAA. Both of these experiments suggest that pCA interfered with normal production of 5HT and 5HIAA and not with the turnover of these compounds. When ^{14}C -5HTP or ^{14}C -tryptophan were given intraventricularly to animals treated 20 hours previously with pCA and the animals were sacrificed 10 to 30 minutes later, ^{14}C -5HT synthesis was not altered after ^{14}C -5HTP was administered but after ^{14}C -tryptophan ^{14}C -5HT content was only 50% of that in animals not treated with pCA. pCA had no effect on the levels of tryptophan present in brain tissues. These results led these authors to speculate that pCA was affecting the synthesis of 5HT from tryptophan but not from 5HTP, suggesting that the enzyme affected was tryptophan hydroxylase.

In a subsequent study Sanders-Bush et al. (1972a) measured the direct effects of pCA on the activity of cerebral tryptophan hydroxylase. Concentrations of pCA up to 10^{-3} M in vitro had no effect on the production of ^{14}C -5HT from ^{14}C -tryptophan. However when the drug was administered 16 hours prior to death a dose related response in tryptophan hydroxylase activity was noted; 2 mg/kg resulted in a 20% reduction of enzyme activity compared to controls, and 10 mg/kg caused a 60% reduction. These results suggested that pCA does inhibit the activity of tryptophan hydroxylase in vivo.

Knapp et al. (1974) described the effects of pCA on tryptophan hydroxylase activity in tissue from rat brain areas containing dense concentrations of 5HT terminals and from areas containing

serotonergic cell bodies. Areas in which cell bodies predominated contained soluble tryptophan hydroxylase and areas in which terminals prevailed contained enzyme localized to the synaptosomal fraction. The fractions chosen for study were the soluble fraction of the midbrain and the synaptosomal fraction of the caudate. They found that 1 hour after administration of pCA (10 mg/kg), striatal synaptosomal tryptophan hydroxylase activity had decreased. Twenty-four hours after drug injection the level of activity was still significantly depressed. Likewise, the activity of soluble enzyme derived from the midbrain was decreased. If pCA were affecting the synthesis only of tryptophan hydroxylase one would not expect such rapid effects on the enzyme activity in the synaptosomal areas.

More recently Koe and Corkey (1976) reported that tryptophan hydroxylase isolated from brainstem was inhibited in vitro by additions of pCA in concentrations greater than 10^{-2} M. In similar systems aromatic amino acid decarboxylase was not inhibited and tyrosine hydroxylase was inhibited but to a lesser degree than was the tryptophan hydroxylase. In animals treated with pCA (15 mg/kg) 17 hours before death, the activity of tryptophan hydroxylase isolated from brainstem was 33% of control whereas tyrosine hydroxylase activity of enzymes isolated from the corpus striatum was virtually the same as controls. In animals treated with pCA and a decarboxylase inhibitor 16 hours prior to making measurements, the accumulation of 5HTP in the midbrain and hindbrain region was only 50% of that of animals treated with the decarboxylase inhibitor only. The tryptophan content of the same brain region was not altered, again suggesting

an effect on the activity of tryptophan hydroxylase. The synthesis of 5HT is decelerated by pCA by some 50% as indicated by the accumulation of 5HTP after a decarboxylase inhibitor. DOPA accumulation in the corpus striatum, in such animals, was decreased to 30% of that in control animals who had received a decarboxylase inhibitor.

Until 1972, only the short term action of pCA had been measured. In 1972 Sanders-Bush et al. (1972a) showed that the greatest decrease in tryptophan hydroxylase activity was at 16 hours after drug treatment, which coincided with the maximum decrease in levels of 5HT and 5HIAA. It was later shown that in rat brain, 5HT, 5HIAA and tryptophan hydroxylase were still significantly reduced 4 months after a single injection of 10 mg/kg pCA (Sanders-Bush et al., 1972b). If pCA treatment was affecting the synthesis of the enzyme such that the molecule itself becomes faulty, restoration of normal enzyme activity would be expected within 2 weeks since this enzyme is completely turned over in this time period (Lovenberg et al., 1967). These authors suggested therefore that the action of pCA was akin to the action of 5,6-dihydroxytryptamine which is neurotoxic to 5HT neurons (Bjorklund et al., 1974).

Fuller and Snoddy (1974) confirmed and extended these observations of Sanders-Bush et al. (1972a). They reported that 4 weeks after a single injection of pCA (20 mg/kg) the tryptophan concentration in brain was unchanged. 5HT and 5HIAA levels and 5HT turnover were all 50% of control. Doses of 50, 100 and 200 mg/kg of tryptophan 1 hour before death resulted in a dose related increase in brain 5HT similar to increases observed after tryptophan loading

in control animals. These results suggest that the existing synthetic mechanisms necessary for 5HT synthesis are not affected by pCA.

Increases in cerebral tryptophan concentrations following a tryptophan load were similar in drug treated and control animals showing that pCA does not affect the uptake of tryptophan into brain tissues.

Fuller and Perry (1974) measured the long term effects of pCA on guinea pig brain 5HT levels. Up to 1 week after a single injection of pCA, levels of 5HT and 5HIAA were still significantly reduced to 50% of control. Because guinea pigs, like mice, metabolize amphetamines mainly by deamination and rats mainly by hydroxylation (see earlier) these results show that a prevention of ring hydroxylation is not the mechanism for the prolonged effect of pCA in brain tissues.

Sanders-Bush et al. (1975) studied in depth this phenomenon of long term reduction of 5-hydroxyindoles. They looked at the effects of this drug on synaptosomal uptake of 5HT, NA and DA when pCA was added in vitro to incubation mixtures of rat brain synaptosomes. The uptake of these transmitters was significantly reduced at concentrations of pCA 2×10^{-7} M or greater. The order of sensitivity to the uptake inhibiting effect of the pCA was 5HT > DA > NA. In rats which had been pretreated with pCA (10 mg/kg) the uptake of 5HT into synaptosomes was markedly reduced. Uptake capacity for 5HT returned very slowly being only 50% of control 90 days after treatment. In contrast the uptake of the catecholamines was only slightly reduced initially and was normal 1 day after injection. When tryptophan hydroxylase activity was measured 1 day after pCA treatment its activity was reduced by 50%. Two weeks after drug injection the level was still 50% lower than control. However, 4 days after drug

treatment the enzyme activity level was only slightly reduced (10 - 28%). The same observations were noted for 5HT synaptosomal uptake but only with small doses of pCA. When 5 mg/kg or greater doses were given there was a marked reduction in the uptake of 5HT at all times. Both 5HT and tryptophan hydroxylase activity were significantly reduced 60 days after a single injection of pCA when the dose exceeded 5 mg/kg.

Levels of 5HT and tryptophan hydroxylase activity, after 10 mg/kg pCA treatment, were measured also in brain regions. 5HT was most greatly reduced in the cerebral cortex and the striatum two weeks after drug treatment. The activity of tryptophan hydroxylase was reduced in all areas at 2 weeks but the most pronounced reduction was seen in the midbrain, hippocampus, cerebral cortex and striatum. At 4 hours the pattern of reduction was similar except that the reduction of tryptophan hydroxylase activity was not as great. Synaptosomes isolated from the brains of treated and control animals showed a greater reduction in 5HT uptake in pCA treated animals than in controls for all areas studied.

Based on these studies the authors concluded that the initial action of pCA could be explained as release of 5HT, inhibition of reuptake, interference with tryptophan hydroxylase or any combination of these. The more prolonged action on tryptophan hydroxylase, synaptosomal uptake and 5HT levels could be explained only by one mechanism, a neurotoxic effect of the pCA. The cerebral cortex and striatum, areas of 5HT nerve terminals, are the most sensitive to pCA. The hypothalamus, another area rich in 5HT nerve terminals

was not as severely affected. The in vivo effects of this drug on the uptake of the catecholamines show that the reduction in synaptosomal uptake of these amines is restricted to periods not exceeding one day.

Gal et al. (1975) have recently described the effects of intraperitoneal injections of pCA on the concentration of 5HT in various areas of the brain. Three days after an intraperitoneal injection of pCA (10 mg/kg) the tryptophan hydroxylase activity in the brainstem was not affected, but in two areas, the hippocampus and pontine area, this enzyme activity was reduced by 40%. After two weeks there was a reduction of enzyme activity in all areas studied except the septum and the cerebellar cortex. Tryptophan hydroxylase was isolated and purified from animals which had been treated two weeks before with pCA and from control animals. The amount of enzyme isolated from drug treated animals was only 40% of that isolated from control animals. Estimates of 5HT turnover in rats receiving the drug were 40% lower than in control animals in the brainstem and telencephalon. Consistent with a neurotoxic effect, pCA caused a long term reduction in the high affinity uptake of 5HT into synaptosomes. Based on these results these authors suggested that the action of pCA may have two different phases: the first, a short term effect lasting for approximately 48 hours; the second a long term effect, possibly resulting from a cytotoxic action.

In this light, Fuller et al. (1975) have reported that the effects of pCA on 5HT depletion is reversible initially but becomes irreversible within 24 to 48 hours. It appears that pCA is taken up by the membrane pump and that continual reuptake is necessary for its

action to be maintained, since the action of pCA can be terminated when a selective uptake blocker is administered shortly after drug treatment.

The idea that pCA may be exerting its effects on serotonergic neuronal systems by some neurotoxic action received further support from the work of Harvey et al. (1975). Histological evidence of cytological damage was noted in Nissl and silver stained sections of rat midbrain areas. Damage was noted in as short a period as 1 day and after as little as 2.5 mg/kg pCA. The cells most affected were cell bodies of the central midbrain corresponding to the B9 cell group as defined by Dahlstrom and Fuxe (1964). After Nissl staining the cells displayed intense staining of the cytoplasm, cellular shrinkage and perineuronal spaces. The effects on the B9 cell group were evident for at least 30 days after pCA treatment. Doses of 5, 10 or 20 mg/kg also produced some visible effects in the B8 and B7 raphe cell groups. The effects though were only modestly severe when compared to the effects on cells in the B9 area. Twenty mg/kg pCA produced a slight neurotoxic effect in the substantia nigra one day after injection, but after 30 days there was no observable effect in this area. The silver stain used in this experiment is capable of demonstrating affected nerve terminals and degenerating axons. There was no evidence that pCA caused this type of damage in the tissues looked at. From 1 to 30 days after pCA treatment glial cells were heavily stained.

Bertilsson et al. (1975) using biochemical techniques, measured the 5HT content in the B7, B8 and B9 cell bodies in animals who had been treated with 5 mg/kg pCA. Analysis was conducted using mass fragmentography. At each time period studied, 0.66 days, 6 days,

14 days and 21 days after pCA treatment, there was no significant reduction in levels of 5HT in the B7, and B8 cell group. At 6 and 14 days after pCA injection 5HT levels in the B9 group were significantly reduced. These results show that at these time periods the B9 cell group is selectively depleted of 5HT by pCA.

Neckers et al.(1975) reported the biochemical effects of an injection of pCA (17 mg/kg) on tryptophan hydroxylase activity in the raphe nuclei and various other areas in the rat brain. Enzyme activity in the dorsal raphe (B7) and the lateral cell group (B9) decreased during the first week after injection. The activity in the dorsal raphe (B7) returned to normal within 2 weeks while that in the B9 was still significantly reduced after 2 months. The activity in the B8 cell group was not reduced by the drug. Reduction in terminal tryptophan hydroxylase activity was greatest in the hippocampus (80 - 92%) followed by the caudate (67 - 73%) then the septum (26 - 44%). After 2 months the enzyme activity in the septum was approaching normal whereas in the caudate and the hippocampus there was little or no increase in the activity. Decreases in 5HT concentrations closely paralleled the enzyme activity in all areas studied.

Meek and Bertilsson (1975) compared the effects of lesioning the B9 cell group by radio-frequency, to the administration of pCA (20 mg/kg) on tryptophan hydroxylase activity and 5HT content in various areas of the rat brain. pCA caused a significant reduction in this enzyme activity and in 5HT content in the hippocampus, the medial geniculate body and the superior colliculus. In the caudate and the substantia nigra, 5HT content was significantly reduced whereas

enzyme activity was not. There was no significant depletion of tryptophan hydroxylase activity after B9 lesions in any of the areas studied. This data suggests that the depletion of enzyme seen in the forebrain after pCA is not due to destruction of the B9 cell group.

Finally, Massari and Sanders-Bush (1975) measured the effects of electrolytic lesions in the B9 cell group, on the synaptosomal uptake of 5HT and compared this to the effects of pCA (15 mg/kg). Two weeks after lesioning or drug injection synaptosomal fractions were isolated from different brain regions. Following lesioning, 5HT uptake was reduced by 35 - 37% in portions of the metathalamus-thalamus, whereas in all other areas the uptake was not significantly reduced. After pCA, 5HT uptake was reduced by 75% or more in all areas studied including the metathalamus-thalamus, the cortex-hippocampus an area consisting of the entire telencephalon, the hypothalamus and the striatum.

These latter two studies show that the prolonged effects of pCA on the synaptosomal uptake of 5HT, tryptophan hydroxylase activity and 5HT content of different brain areas are not entirely due to an effect of pCA on the B9 cell group. Projections from the B9 cell group are not entirely known. From the work of Massari and Sanders-Bush (1975) and that noted under the histochemical distribution of 5HT, serotonergic afferents arising from the B9 cell group have been noted to project into the caudate and the metathalamus-thalamus. pCA appears to affect most areas in the rat brain which contain 5HT terminals.

Table 4 gives a summary of the reported effects of pCA on 5HT in CNS tissues. From all of the data presented to date it appears

likely that this drug exerts a neurotoxic effect mainly in the 5HT terminal areas and in the B9 cell group. It does not appear that destruction of this cell group is the sole cause of reduction of 5HT in terminal areas.

Table 4 Summary of effects of pCA on cerebral amines.

<u>Action</u>	<u>References</u>	<u>Reported Work</u>
Inhibition of uptake	Morgan et al., 1971	slices; NA and 5HT
	Costa and Revuelta, 1972	whole brain; 5HT
	Wong et al., 1973	synaptosomes; 5HT and NA
	Sanders-Bush et al., 1975	synaptosomes; 5HT, NA and DA <u>in vitro</u>
		synaptosomes; 5HT long term <u>in vivo</u>
Release of transmitter	Gal et al., 1975	synaptosomes; 5HT
	Massari and Sanders-Bush, 1975	synaptosomes; 5HT
	Miller et al., 1970	indirect evidence for 5HT and NA release
	Strada and Sulser, 1971	NA in hypothalamus
	Wong et al., 1973	5HT from synaptosomes
MAO inhibition	Leonard, 1976	NA, DA and 5HT release increased
	Pletscher et al., 1964	indirect evidence
	Fuller and Hines, 1965	indirect evidence
	Strada et al., 1970	reduction of deaminated products of NA
	Fuller and Hines, 1970	reduction of 5HIAA from a ³ H-5HT load
	Morgan et al., 1972	measured directly

Table 4 Summary of effects of PCA on cerebral amines (cont) .

<u>Action</u>	<u>References</u>	<u>Reported Work</u>
Turnover	Costa et al., 1971	decreased turnover of DA in corpus striatum
	Costa and Revuelta, 1972	turnover of 5HT slower
	Fuller and Snoddy, 1974	turnover of 5HT reduced
	Gal et al., 1975	turnover of 5HT reduced
Synthesis of 5HT inhibition	Sanders-Bush and Sulser, 1970	inhibition of tryptophan hydroxylase
	Sanders-Bush et al., 1972 a,b	inhibition of tryptophan hydroxylase
	Knapp et al., 1974	inhibition of tryptophan hydroxylase
	Sanders-Bush et al., 1975	inhibition of tryptophan hydroxylase
	Neckers et al., 1975	inhibition of tryptophan hydroxylase
	Meek and Bertilsson, 1975	inhibition of tryptophan hydroxylase
	Koe and Corkey, 1976	inhibition of tryptophan hydroxylase
Neurotoxicity	Harvey et al., 1975	histological cytodestruction
	Bertilsson et al., 1975	biochemical assessment of cell body areas

(2.) DESIGN OF THIS STUDY

This study was designed to examine the effects of a single injection of pCA on the levels of DA, NA and 5HT in four different areas of the rat brain. These areas included the mesencephalon-pons which contains cell bodies responsible for the major rostral projections to areas of terminal DA, NA and 5HT, the diencephalon, an area rich in terminal NA, the corpus striatum an area rich in DA terminals and the hippocampus an area rich in 5HT terminals.

The effects of this single injection of pCA were to be studied 3 days and 30 days after treatment when the animals were sacrificed immediately or given a tryptophan load one hour before death. The study was therefore a factorial design (Table 5) and was analyzed according to this using analysis of variance. The main effects to be included were pCA, tryptophan loading and time.

Table 5 Schematic representation of the factorial design used in this experiment.

no pCA				pCA			
no tryptophan		tryptophan		no tryptophan		tryptophan	
3 day	30 day	3 day	30 day	3 day	30 day	3 day	30 day

Eight different groups were to be studied. Results from the analysis of variance were to be used to determine where the statistical significance occurred between the various groups. When statistical significance was found after the analysis of variance the data was to be further analyzed to determine which of the components within the groups were significant. It was decided that since there was non-orthogonal data in some groups, "t" tests would be used to do the final analyses.

(3.) MATERIALS AND METHODS

(3.1) Materials and instruments

Appendices A - C list the chemicals, equipment and various solutions used in this study.

(3.2) Methods

(3.2.1) Drug treatments

Male Wistar rats (Woodland Farms, Ontario) were used in this experiment and ranged in weight from 140 - 200 gms at the beginning of the treatment schedules. All drugs were administered intraperitoneally (i.p.) in volumes not exceeding 1 ml for pCA and 2.0 ml for tryptophan. Controls were given equivalent volume injections of drug vehicles. The D,L form of pCA (Regis Chemicals) was dissolved in physiological saline and administered at 10 mg/kg 3 days or 30 days before death. The L form of tryptophan (Raylo Chemicals) was dissolved in a physiological saline 0.5% tween solution and administered at 100 mg/kg 1 hour before death.

Four groups of animals were studied. The first and second groups represented the short-term treatment groups. Group 1 received pCA treatment 3 days before death, with controls receiving vehicle injections 3 days before death. Group 2 received pCA treatment 3 days before death, with an added tryptophan treatment 1 hour before death. Controls received pCA vehicle 3 days before death, with an added tryptophan treatment, as in the drug group. Groups 3 and 4 represented the long term treatment groups. Group 3 received pCA injections 30 days before death, while controls received drug vehicle 30 days before death. Group 4 received pCA injections 30 days before death

along with an injection of tryptophan one hour before death. Controls in this group received injections of pCA vehicle 30 days before death with tryptophan treatment, as in the drug group. At least 16 animals were used in each of the four groups. A pooled tissue sample from two rat brains was used for each analysis. The sample size for drug and control subgroups was therefore at least 4.

For 24 hours after pCA injections animals were housed one to a cage in a room in which the temperature was at or below 20°C. This procedure was necessary to guard against loss of animals due to extreme hyperthermia produced by the drug. Thereafter, animals were returned to the colony room where they were housed two to a cage for the duration of the experiment. Throughout the experiment they received food and water ad libria. Photoperiods were controlled at 12 hours dark and 12 hours light. Drugs were administered such that animals were always sacrificed between 1500 and 1600 hours. Animals were sacrificed by decapitation under light ether anaesthesia and their brains were dissected immediately.

(3.2.2) Dissection techniques

The surface of the skull was exposed through a midline incision along the surface of the scalp. The skin was peeled back exposing the full margin of the skull below. A transverse cut was then made through the muscle tissue surrounding the occipital bone to the level of the foramen magnum. Scissors were then inserted into the foramen and the occipital bone was cracked to the surface of the skull, where additional upward pressure was applied to break through the inter-parietal bone and separate the sagittal suture. Using forceps the

parietal bone was broken away at the coronal suture and then removed. Further chipping from this break allowed for removal of sufficient frontal bone to expose the brain below. Lateral areas of the skull were further chipped and the tympanic horns broken to allow complete access to the brain. The brain was then lifted from the cranium with the olfactory bulbs left behind. The optic nerve, optic chiasma and other cranial nerves were severed as the brain was lifted free of the skull.

Once the whole brain had been removed, the pineal gland was removed from its site at the junction of the longitudinal and transverse fissures and discarded. Next, a sagittal cut was made through the vermis of the cerebellum to the floor of the fourth ventricle. This tissue mass was then removed from the brainstem by cuts through the middle and inferior penduncles and discarded.

Following this procedure the two cerebral hemispheres were pressed apart at the longitudinal fissure and the corpus callosum was cut. The right and left hemispheres were then peeled back exposing the roof or tectum of the midbrain and the diencephalon which was covered by the hippocampus. The anterior parts of the hemispheres were cut away from the rest of the brain and the two tissue masses removed for further dissection. The corpus striatum (containing the caudate nucleus, the putamen and the globus pallidus) was dissected free of the cerebral hemispheres. This complex, which comes attached to the ventral surface of the cerebral hemisphere, is separated from it by the external capsule and the corpus callosum. Medially this area was separated from the diencephalon by the lateral ventricle.

These anatomical structures were used to outline the extent of this 'area' and it was literally pinched free of the hemisphere using fine forceps.

Next the hippocampus was removed from the diencephalon below by making a small incision through the hippocampal fissure and separating each half by peeling it laterally. Both the hippocampus and the gyrus dentatus were removed and included in the measurements for this area.

The whole of the mesencephalon-pons was separated together and included as one area by first making a transverse cut through the area where cranial nerves IX and X exit from the brainstem. This separates the pons area from the medulla oblongata lying caudal to it. A second transverse cut between the anterior colliculus and the medial geniculate bodies separates the diencephalon from the mesencephalon. The diencephalon was dissected free of the septum, which extends rostrally, and the pyriform cortex. This area included the thalamus, the hypothalamus, the epithalamus or area lying in the dorsal midline region from the thalamus, the metathalamus which lies on the dorsolateral boundary of the thalamus containing both the lateral and medial geniculate bodies and the subthalamus which lies ventrolateral to the thalamus.

The above areas were dissected free within 5 - 8 minutes and immediately frozen in isopentane cooled to the temperature of frozen carbon dioxide. Tissues were then placed in an ultra low temperature freezer and stored at minus 60°C until they were measured for amine content. Extractions were made within seven days of dissection by the following biochemical techniques.

(3.2.3) Biochemical techniques

(3.2.3.1) Extraction procedures

The extraction of all three amines was conducted according to the following procedure which is a modification of that of Cox and Perhach (1973). Each measurement was conducted on combined tissue from two rat brains. The tissue was removed from the freezer and the weight was recorded. The tissue was then minced and transferred to a glass mortar to which 6 ml of ice-cold acidified butanol was added. The homogenizer was adjusted to a speed of 4 and the tissue homogenized using a motor driven teflon pestle. The homogenate was transferred to a 16 x 100 mm centrifuge tube and spun in a fixed angle rotor at 3000 rpm for 5 minutes. 2.5 ml of the supernatant was then transferred to each of two 50 ml glass stoppered centrifuge tubes which contained 2.5 ml of 0.01N HCl and 5 ml of heptane. One tube of the pair was used to determine recovery and to it was added 1 μ l of each of the 3 standard solutions (DA, NA and 5HT). Thereafter both tubes were treated identically, according to the following scheme.

The tubes were vigorously shaken for 5 minutes on a mechanical shaker and then centrifuged at 1500 rpm for 10 minutes. 2.4 ml of the acid layer was removed and transferred to a 16 x 100 mm test tube containing 200 mg of alumina and 1.0 ml of 2M sodium acetate. These tubes were covered with paraplasm film and gently shaken on a mechanical shaker for 10 minutes. They were then removed and centrifuged at 3000 rpm for 5 minutes. 3 ml of the resulting supernatant was removed and added to a 50 ml tube containing 3 gm of sodium chloride, 1 ml of borate buffer (pH 10) and 6 ml of butanol.

The mixture was placed on a mechanical shaker and shaken vigorously for 10 minutes, then centrifuged at 1500 rpm for 10 minutes. 5 ml of the butanol phase was removed and added to a tube containing 6 ml of heptane and 0.5 ml of 0.1N HCl. The 5HT was extracted into the acid phase by shaking this mixture vigorously for 5 minutes on the mechanical shaker. After centrifuging again at 1500 rpm for 10 minutes, 0.4 ml of the acid phase was transferred to a 13 x 100 mm tube for further analysis. The remainder of the aqueous above the alumina was aspirated off and the catecholamines were extracted from the gel. The alumina was washed by shaking it gently for 5 minutes with 2.0 ml of distilled water. This was followed by a centrifugation at 3000 rpm for 5 minutes. The water was discarded and replaced with 2.0 ml of 0.1N acetic acid. The tube was then shaken gently on a mechanical shaker for 10 minutes and centrifuged at 3000 rpm for 5 minutes. 1.0 ml of the acid layer was transferred to each of two 13 x 100 mm test tubes for analysis. One tube was used for analysis of the amine content; the other was used to estimate the tissue blank.

(3.2.3.2) Analysis of amines

Both the catecholamines and 5HT were analyzed according to the following procedure which was adapted from Cox and Perhach (1973). 5HT was converted to an intense fluorophore by the addition of 0.6 ml o-phthalaldehyde reagent to 0.4 ml of acid phase obtained as indicated above. A reagent blank was formed by reacting 0.6 ml of the o-phthalaldehyde reagent with 0.4 ml of 0.1N HCl. Once the tubes had been thoroughly mixed, they were covered by paraplax film and a small hole was punched in the top with a 21 gauge needle. The tubes were then placed in a boiling water bath for 15 minutes, after which

they were removed and rapidly brought to room temperature in a cold water bath. Measurements were made in an Aminco Bowman spectrophotofluorometer at an excitation wavelength of 360 nm and emission wavelength of 470 nm.

The catecholamines were converted into fluorophores by the following steps. First, 0.2 ml of EDTA reagent was added to the acid extract from the alumina and the pH was adjusted to between 6.3 and 6.8. Care was taken to ensure that the pH of all the samples measured for each extraction were identical. Second, 0.1 ml of iodine reagent was added, the tubes mixed thoroughly and left to stand for exactly 2 minutes. Third, 0.2 ml of the alkaline sulfite reagent was added, the tubes were mixed again and left to stand for an additional 2 minutes. Fourth, 0.2 ml of 5N acetic acid was added. The tubes were then left to stand for 2 minutes, following which they were placed in a boiling water bath for exactly 2 minutes. At the end of this time the tubes were removed and rapidly cooled to room temperature in a cold water bath. Fluorescence due to NA was measured at this point in a spectrophotofluorometer with activation and emission settings of 385 nm and 485 nm respectively. The DA fluorophore was generated by placing the tubes back into the boiling water bath for a further 4 minutes after which they were removed, cooled to room temperature and measured in a spectrophotofluorometer at an excitation setting of 320 and an emission setting of 370 nm. Tissue blanks were prepared by the following procedure. 0.2 ml of alkaline sulfite was added to the acid - EDTA mixture and left to

stand for 2 minutes. 0.1 ml of the iodine reagent was added and the mixture again left for 2 minutes. 0.2 ml of 5N acetic acid was then added and the mixture was again left for 2 minutes. The tubes were then placed in a boiling water bath for exactly 2 minutes, removed and cooled to room temperature. Measurements were taken at the excitation and emission wavelengths for NA. The tubes were then returned to the boiling water bath and left for an additional 4 minutes. DA tissue blanks were then measured at the excitation and emission wavelengths for DA.

The settings of the Aminco Bowman Spectrophotofluorometer were consistent for each of the measurements made.

(3.2.4) Aminco Bowman Instrumentation

An Aminco Bowman Spectrophotofluorometer was used in this experiment to measure the fluorescence produced by the samples. In this instrument ultraviolet light is generated from a high intensity Xenon continuum light source. This light is passed into an excitation monochromator where a particular monochromatic wavelength is selected from the Xenon spectrum and focused onto the sample cell. Upon entering the sample cell compartment, the excitation light passes through slit 2 which controls the band width of the wavelengths. Slit 1 is situated at the light source and acts as a baffle and as such is not adjustable. Slit 3 sits between slit 2 and the cell where it blocks stray light originating from the edges of slit 2 thus preventing interference from reflected light.

Similarly, fluorescent light from the sample is directed into an emission monochromator after passing through slits 4 and 5. From here

it is directed onto the photomultiplier tube through slit 6 which is nothing more than a shutter and slit 7 which is attached to the photomultiplier tube housing. Slit 7 is adjustable and controls the band width of light reaching the photomultiplier tube.

The slit positions used in this experiment were as follows

Slit number	1	2	3	4	5	6	7
Slit size in mm	-	2	2	2	2	-	5

The energy which strikes the photomultiplier tube is then transferred to a photomultiplier microphotometer and the relative intensity of fluorescence generated by the sample can be read from the meter on this instrument. The sensitivity of the microphotometer was adjusted to 40 which results in a 1.4 times increase in the voltage going to the meter.

Samples were measured at the appropriate settings for each of the amines studied. Serotonin was measured at an excitation of 360 nm and emission of 470 nm. Noradrenaline was measured at an excitation wavelength of 385 nm and emission of 485 nm while dopamine was measured at an excitation of 320 nm and an emission of 370 nm. Each of these wavelengths can be selected by the wavelength selectors attached to each of the monochromators.

(3.2.5) Calculations

For each measurement three readings were obtained. The first reading, called the test, gave the fluorescence of the sample due to the endogenous amine levels. The second reading, called the recovery, gave the fluorescence value of the sample which was due to both the endogenous amine and an added known amount of authentic

amine. The third reading was of a tissue blank for the catecholamine analysis as described in the section on analysis techniques, or a reagent blank for the 5HT analysis which was assumed to equal tissue blanks.

The levels of endogenous amine were then calculated by the following formulas:

$$F_{r-b} - F_{t-b} = Fa$$

$$X = \frac{F_{t-b}}{Fa} (a)$$

with

F_{r-b} = fluorescence due to recovery minus blank

F_{t-b} = fluorescence due to test minus blank

Fa = fluorescence due to added authentic amine

a = known amount of amine added

X , then was the amount of endogenous amine present in 2.5 ml of the initial homogenate. This value was then extrapolated to find the amount present in 6 ml of the original homogenate. The amount of endogenous amine present in this tissue extract was then expressed as the amount present in one gram of tissue.

(4.) RESULTS

The raw data obtained from this study can be found in appendices D - K. A summary of this data showing the means and standard deviations obtained for DA, NA and 5HT in each of the groups studied are found in appendices L - N.

The following sections will discuss the results obtained for individual amines in each of the four areas studied. The changes noted in each of the experimental groups will be presented first, followed by changes noted when results obtained after 3 days are compared to those obtained after 30 days. The results for the analyses of variance will then be given followed by the results obtained for "t" tests.

(4.1) The effects after 3 and 30 days of tryptophan loading, pCA and pCA plus tryptophan loading on DA, NA and 5HT in the mesencephalon.

(4.1.1) Changes in DA levels

Table 6 and Fig. 9 show the effects of all treatments after 3 and 30 days on the levels of DA in the mesencephalon.

In animals measured after 3 days the levels of DA increased after a tryptophan load by 23% when compared to controls. In pCA treated animals the levels of DA decreased by 15% when compared to controls. In animals who were given pCA plus a tryptophan load there was a 10% decrease in the levels of DA when this group was compared to controls (i.e. no pCA or tryptophan) but there was a 6% increase when compared to pCA treated animals without a tryptophan load. These changes are shown in Table 6. None of these changes were statistically significant according to "t" tests as will be discussed in detail later.

In animals measured after 30 days the levels of DA decreased after a tryptophan load by 36% when compared to controls. In pCA treated animals the levels of DA decreased by 40% when compared to controls. In animals who were given pCA plus a tryptophan load, there was a 45% decrease in the levels of DA when this group was compared to controls (i.e. no pCA or tryptophan) and a 8% decrease when compared to pCA treated animals without a tryptophan load. These changes are shown in Table 6. None of these changes were statistically significant according to "t" tests as will be discussed later in detail.

(4.1.2) Changes in NA levels

Table 7 and Fig. 10 show the effects of all treatments after 3 and 30 days on the levels of NA in the mesencephalon.

In animals measured after 3 days the levels of NA increased after a tryptophan load by 16% when compared to controls. In pCA treated animals, the levels of NA decreased by 1% when compared to

Table 6 The percentage and direction of change for DA in the mesencephalon for each of the four comparisons made. Controls were given pCA vehicle injection or tryptophan vehicle injection at times equivalent to treatment with these compounds. Tryptophan was administered 1 hour before death. pCA was administered either 3 days or 30 days before death. Statistical testing with "t" tests showed no significance.

	3 day	30 day
Control vs try	23% ↑	36% ↓
Control vs pCA	15% ↓	40% ↓
Control vs pCA + try	10% ↓	45% ↓
pCA vs pCA + try	6% ↑	8% ↓



Fig. 9 Mean values of DA levels in mesencephalon showing the effects of tryptophan loading (try), pCA treatment (pCA) and a combined treatment (pCA + try). One standard deviation from the mean is represented by the bars. Both 3 and 30 day groups are shown.

Table 7 The percentage and direction of change for NA in the mesencephalon for each of the four comparisons made. Controls were given pCA vehicle injection or tryptophan vehicle injection at times equivalent to treatment with these compounds. Tryptophan was administered 1 hour before death. pCA was administered either 3 days or 30 days before death. Statistical testing with "t" tests showed no significance.

	3 day	30 day
Control vs try	16% ↑	2% ↑
Control vs pCA	1% ↓	-
Control vs pCA + try	12% ↑	15% ↑
pCA vs pCA + try	13% ↑	15% ↑

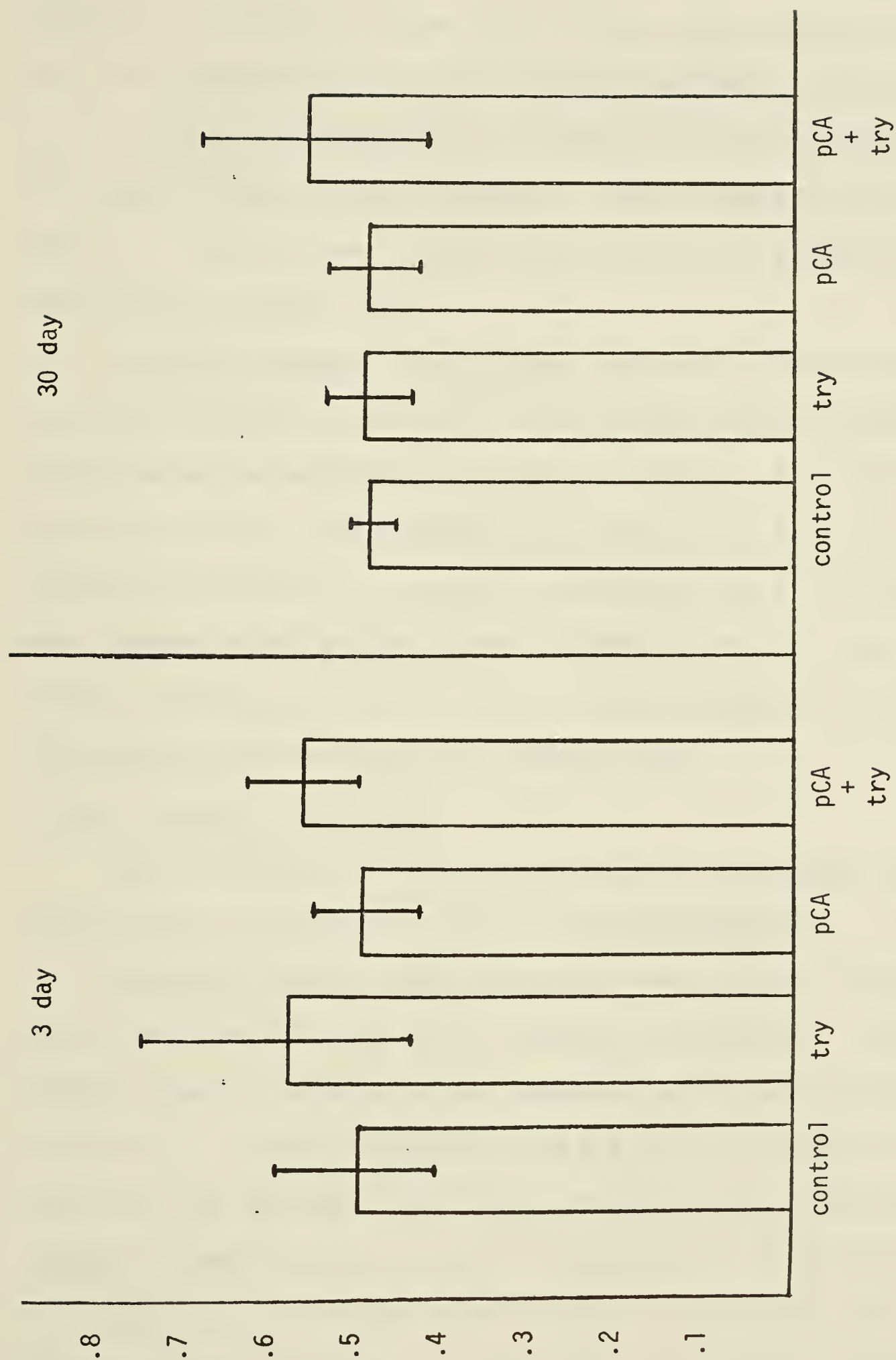


Fig. 10 Mean values of NA levels in the mesencephalon showing the effects of tryptophan loading (try), pCA treatment (pCA) and a combined treatment (pCA + try). One standard deviation from the mean is represented by the bars. Both 3 and 30 day groups are shown.

controls. In animals who were given pCA plus a tryptophan load, there was a 12% increase in the levels of NA when compared to controls (i.e. no pCA or tryptophan) and an increase of 13% when compared to pCA treated animals without tryptophan. These changes are shown in Table 7 . None of these changes were statistically significant as will be discussed in detail later.

In animals measured after 30 days the levels of NA increased by 2% when compared to controls. In pCA treated animals, the levels of NA remained unchanged when compared to controls. In animals who were given pCA plus a tryptophan load, there was a 15% increase when compared to controls (i.e. no pCA or tryptophan) and a 15% increase when compared to pCA treated animals without a tryptophan load. These changes are shown in Table 7 . None of these changes were statistically significant as will be discussed in detail later.

(4.1.3) Changes in 5HT levels

Table 8 and Fig. 11 show the effects of all treatments after 3 and 30 days on the levels of 5HT in the mesencephalon.

In animals measured after 3 days the levels of 5HT increased after a tryptophan load by 31% when compared to controls. In pCA treated animals, the levels of 5HT decreased by 47% when compared to controls. In animals who were given pCA plus a tryptophan load there was a 24% decrease in the levels of 5HT when this group was compared to controls (i.e. no pCA or tryptophan) but a 44% increase when compared to pCA treated animals without a tryptophan load. These changes are shown in Table 8 . There was a statistically significant difference between the pCA treated animals and controls ($p < .01$), which will be described in more detail later.

Table 8 The percentage and direction of change for 5HT in the mesencephalon for each of the four comparisons made. Controls were given pCA vehicle injection or tryptophan vehicle injection at times equivalent to treatment with these compounds. Tryptophan was administered 1 hour before death. pCA was administered either 3 days or 30 days before death. Statistical significance according to "t" tests is noted.

	3 day	30 day
Control vs try	31% ↑	38% ↑
Control vs pCA	§ 47% ↓	** 40% ↓
Control vs pCA + try	24% ↓	8% ↓
pCA vs pCA + try	44% ↑	§§ 53% ↑

§ Statistically significant $p < .01$

** Statistically significant $p < .005$

§§ Statistically significant $p < .001$

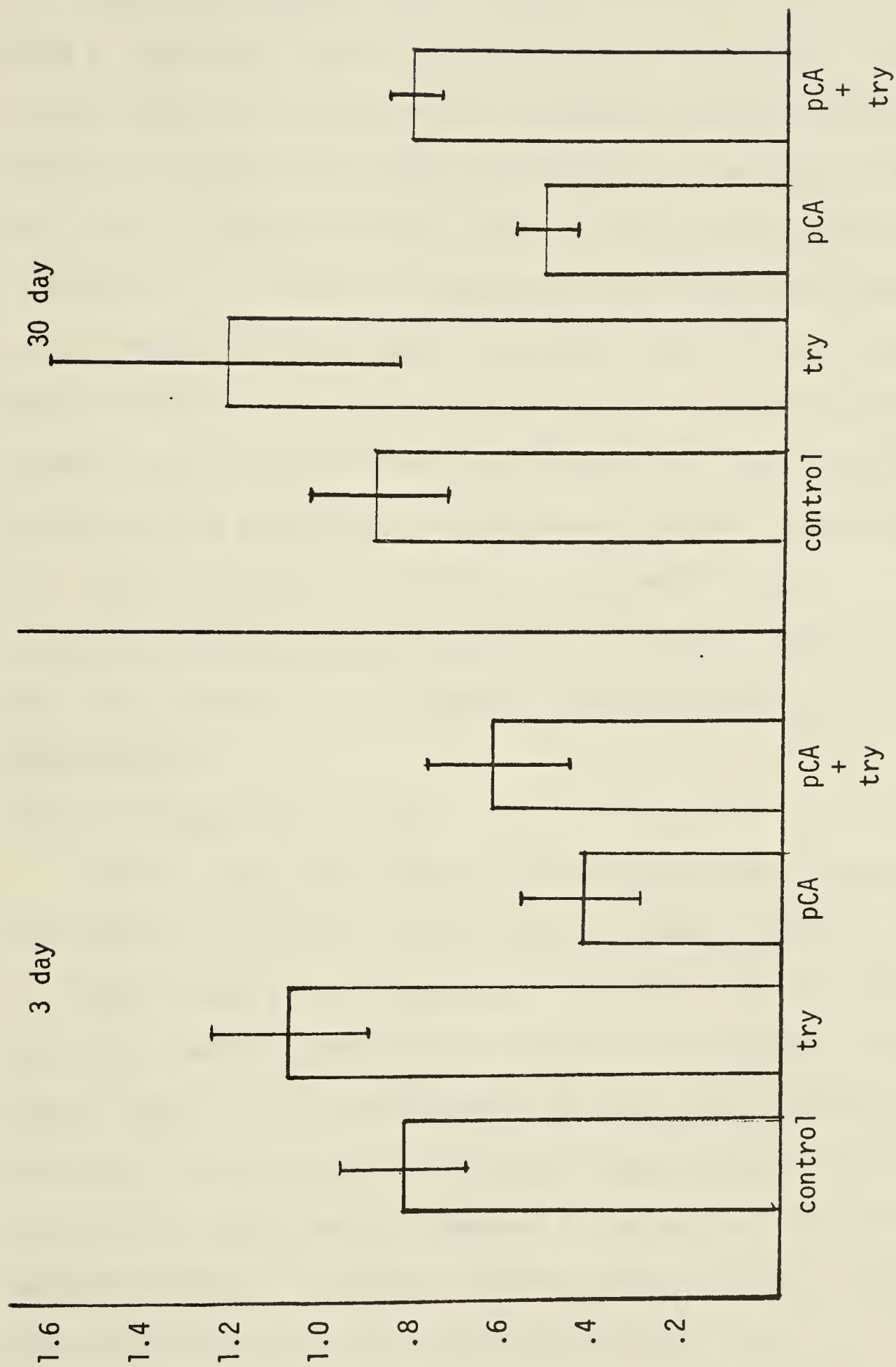


Fig. 11 Mean values of 5HT levels in mesencephalon showing the effects of tryptophan loading (try), pCA treatment (pCA) and a combined treatment (pCA + try). One standard deviation from the mean is represented by the bars. Both 3 and 30 day groups are shown.

In animals measured after 30 days the levels of 5HT increased after a tryptophan load by 38% when compared to controls. In pCA treated animals the levels of 5HT decreased by 40% when compared to controls. In animals who were given pCA plus a tryptophan load there was a 8% decrease in the levels of 5HT when this group was compared to controls (i.e. no pCA or tryptophan) but a 53% increase when compared to pCA treated animals without a tryptophan load. These changes are shown in Table 8 . There was a statistically significant difference between the pCA treated animals and controls ($p < .005$) and between the pCA treated animals and the pCA plus tryptophan loaded animals ($p < .001$) which will be described in more detail later.

(4.2) The effects of measuring amines at different times (3 or 30 days after treatment) on the levels of DA, NA and 5HT in the mesencephalon.

(4.2.1) Changes in DA levels

Table 9 shows the effects of time on the levels of DA in the mesencephalon for each of the experimental groups studied.

When the levels of DA measured in control animals 3 days or 30 days after control injections are compared to one another, the levels were 92% higher in the group measured 30 days after injection. When the levels of DA measured in tryptophan loaded animals 3 days or 30 days after control injections are compared to one another, the levels remained constant. The levels of DA in animals treated 30 days prior with pCA were 37% higher than the levels obtained from animals measured 3 days after pCA treatment. The levels of DA in animals given a tryptophan load 30 days after pCA treatment were 18% higher than those in animals given a tryptophan load 3 days after a pCA treatment. None

Table 9 The percentage and direction of change for the differences in amine levels noted in the mesencephalon between animals measured 3 days after treatment and animals measured 30 days after treatment. Controls were given pCA vehicle injection or tryptophan vehicle injection at times equivalent to treatment with these compounds. Tryptophan was administered 1 hour before death. pCA was administered either 3 days or 30 days before death. Statistical testing with "t" tests showed no significance.

		Control	try	pCA	pCA + try
DA	3 day vs 30 day	92% ↑	-	37% ↑	18% ↑
NA	3 day vs 30 day	3% ↑	15%↓	2% ↓	-
5HT.	3 day vs 30 day	8% ↑	14%↑	22% ↑	29% ↑

of these differences are statistically significant as will be discussed in detail later.

(4.2.2) Changes in NA levels

Table 9 shows the effects of time on the levels of NA in the mesencephalon for each of the experimental groups studied.

When the levels of NA measured in controls animals 3 days or 30 days after controls injections are compared to one another, the levels were 3% lower in the group measured 30 days after injection. When the levels of NA measured in tryptophan loaded animals 3 or 30 days after control injection are compared to one another, the levels were 15% lower in the group measured 30 days after injection. The levels of NA in animals treated 30 days prior with pCA were 2% lower than the levels obtained from animals measured 3 days after pCA treatment. The levels of NA in animals given a tryptophan load 30 days after pCA treatment were the same as those in animals given a tryptophan load 3 days after a pCA treatment. None of these differences were statistically significant as will be discussed in detail later.

(4.2.3) Changes in 5HT levels

Table 9 shows the effects of time on the levels of 5HT in the mesencephalon for each of the experimental groups studied.

When the levels of 5HT measured in control animals 3 days or 30 days after control injections are compared to one another, the levels were 8% higher in the group measured 30 days after injection. When the levels of 5HT measured in tryptophan loaded animals 3 days or 30 days after control injection are compared to one another, the levels were 14% higher in the groups measured 30 days after injection. The levels of 5HT in animals treated 30 days prior with pCA were 22%.

higher than the levels obtained from animals measured 3 days after pCA treatment. The levels of 5HT in animals given a tryptophan load 30 days after pCA treatment were 29% higher than those in animals given a tryptophan load 3 days after a pCA treatment. None of these differences were statistically significant, as will be discussed in detail later.

(4.3) Results of analysis of variance done on the amounts of individual amines in the mesencephalon.

An analysis of variance of DA in the mesencephalon reveals that treatment with pCA had a statistically significant effect on the levels of this amine in this area of the brain ($\alpha = .025$). Time also had a statistically significant effect on the levels of this amine in the mesencephalon ($\alpha = .025$). Results of this analysis can be seen in Table 10 .

An analysis of variance of NA in the mesencephalon reveals that there is no statistically significant difference in the effects of either pCA, tryptophan or time on NA in this area of the brain. The results of this analysis can be seen in Table 11 .

Analysis of variance of 5HT in the mesencephalon reveals that tryptophan loading and treatment with pCA had statistically significant effects on the levels of this amine in this area of the brain ($\alpha = .005$ in both cases). Time also had a statistically significant effect on the levels of this amine in the mesencephalon ($\alpha = .05$). Results of this analysis can be seen in Table 12 .

(4.4) Results of "t" tests done on individual groupings within the mesencephalon where significance was noted after the analysis of variance.

Table 10 Results of analysis of variance done for DA in the mesencephalon.

Source	DF	SS	MS	F	Level of Significance
pCA	1	.021336	.021336	7.109312	.025
try	1	.002465	.002465	.821454	n.s.
pCA x try	1	.001885	.001885	.628090	n.s.
time	1	.020435	.020435	6.80907	.025
pCA x time	1	.002049	.002049	.682682	n.s.
try x time	1	.012160	.012160	4.051849	n.s.
pCA x try x time	1	.006256	.006256	2.084624	n.s.
Error	23	.069027	.003001		
Total	30	.135615			

Table 11 Results of analysis of variance done for NA in the mesencephalon.

Source	DF	SS	MS	F	Level of Significance
pCA	1	.009790	.009790	.141073	n.s.
try	1	.023302	.023302	3.357120	n.s.
pCA x try	1	.001184	.001184	.170583	n.s.
time	1	.005513	.005513	.794193	n.s.
pCA x time	1	.003902	.003902	.562210	n.s.
try x time	1	.001709	.001709	.246190	n.s.
pCA x try x time	1	.002970	.002970	.427945	n.s.
Error	23	.159648	.006941		
Total	30	.199208			

Table 12 Results of analysis of variance done for 5HT in the mesencephalon.

Source	DF	SS	MS	F	Level of Significance
pCA	1	1.291139	1.291139	56.424217	.005
try	1	.545025	.545025	23.818179	.005
pCA x try	1	.005817	.005817	.254201	n.s.
time	1	.115052	.115052	5.027904	.05
pCA x time	1	.002840	.002840	.124101	n.s.
try x time	1	.016002	.016002	.699288	n.s.
pCA x try x time	1	5.633×10^{-7}	5.633×10^{-7}	2.462×10^{-5}	n.s.
Error	23	.526302	.022883		
Total	30	2.502177			

"t" tests done on the effects of pCA treatment on the levels of DA in the mesencephalon are shown in Table 13 .

Table 13 Results of "t" tests for the levels of DA in animals who have been given pCA compared to those who have not been given pCA.

<u>Without</u>	<u>With</u>	<u>t</u>	<u>Level of Significance</u>	<u>Degrees of Freedom</u>
3 day	3 day	1.382	n.s.	6
30 day	30 day	1.839	n.s.	6
3 day	3 day try	.840	n.s.	6
30 day	30 day try	2.099	n.s.	6
3 day try	3 day try	1.698	n.s.	6
30 day try	30 day try	.728	n.s.	5

None of the changes in DA levels after a pCA treatment were statistically significant according to "t" tests.

"t" tests done on the levels of DA at 3 and 30 days in the mesencephalon are shown in Table 14 .

Table 14 Results of "t" tests for the levels of DA comparing animals 3 days after treatment to animals 30 days after treatment.

<u>3 day</u>	<u>30 day</u>	<u>t</u>	<u>Level of Significance</u>	<u>Degrees of Freedom</u>
Control	Control	2.266	n.s.	6
Try	Try	.009	n.s.	5
pCA	pCA	2.158	n.s.	6
pCA + Try	pCA + Try	1.327	n.s.	6

None of the changes in DA levels between animals measured 3 days after treatment compared to those measured 30 days after treatment were statistically significant according to "t" tests.

"t" tests done on the effects of tryptophan on the levels of 5HT in the mesencephalon are shown in Table 15 .

Table 15 Results of "t" tests for the levels of 5HT in animals who have been given tryptophan compared to those who have not been given tryptophan.

<u>Without</u>	<u>With</u>	<u>t</u>	<u>Level of Significance</u>	<u>Degrees of Freedom</u>
3 day	3 day	2.244	n.s.	6
30 day	30 day	2.071	n.s.	5
3 day	3 day pCA	1.989	n.s.	6
30 day	30 day pCA	.997	n.s.	6
3 day pCA	3 day pCA	1.863	n.s.	6
30 day pCA	30 day pCA	7.046	.001	6

In animals who had not been given pCA the changes in 5HT after a tryptophan load were not statistically significant. 3 days after pCA treatment the changes noted in 5HT levels after a tryptophan load were not statistically significant when the levels are compared to animals who had been given pCA, nor when compared to controls (i.e. no pCA or tryptophan). Thirty days after pCA treatment the changes noted in 5HT levels after a tryptophan load were statistically significant when compared to animals who had been given pCA, but were not statistically significant when compared to controls (i.e. no pCA or tryptophan).

"t" tests done on the effects of pCA on the levels of 5HT in the mesencephalon are shown in Table 16 .

Table 16 Results of "t" tests for the levels of 5HT in animals who have been given pCA compared to those who have not been given pCA.

<u>Without</u>	<u>With</u>	<u>t</u>	<u>Level of Significance</u>	<u>Degrees of Freedom</u>
3 day	3 day	4.265	.01	6
30 day	30 day	4.480	.005	6
3 day try	3 day try	3.842	.01	6
30 day try	30 day try	2.960	.05	6

The differences in the levels of 5HT between animals who had been given pCA and those who have not were statistically significant both 3 days and 30 days after pCA treatment. In animals who had been given tryptophan along with pCA the levels of 5HT differed with statistical significance from those who had just been given tryptophan alone at both 3 days and 30 days after treatment.

"t" tests done on the levels of 5HT at 3 and 30 days in the mesencephalon are shown in Table 17 .

Table 17 Results of "t" tests for the levels of 5HT comparing animals 3 days after treatment to animals 30 days after treatment.

<u>3 day</u>	<u>30 day</u>	<u>t</u>	<u>Level of Significance</u>	<u>Degrees of Freedom</u>
Control	Control	.569	n.s.	6
Try	Try	.863	n.s.	5
pCA	pCA	1.319	n.s.	6
pCA + try	pCA + try	2.220	n.s.	6

None of the changes in 5HT levels between animals measured 3 days after treatment compared to those measured 30 days after treatment were statistically significant according to "t" tests.

(4.5)The effects after 3 and 30 days of tryptophan loading, pCA and pCA plus tryptophan loading on DA, NA and 5HT in the diencephalon.

(4.5.1) Changes in DA levels

Table 18 and Fig. 12 show the effects of all treatments after 3 and 30 days on the levels of DA in the diencephalon.

In animals measured after 3 days the levels of DA increased after a tryptophan load by 79% when compared to controls. In pCA treated animals the levels of DA decreased by 3% when compared to controls. In animals who were given pCA plus a tryptophan load, there was no change in the levels of DA when compared to control (i.e. no pCA or tryptophan) but there was a 3% increase when compared to pCA treated animals without a tryptophan load. These changes are shown in Table 18 . The increase noted after a tryptophan load was statistically significant ($p < .05$) as will be discussed in detail later.

In animals measured after 30 days the levels of DA increased after a tryptophan load by 32% when compared to controls. In pCA treated animals the levels of DA increased by 2% when compared to controls. In animals who were given pCA plus a tryptophan load, there was a 25% increase in the levels of DA when this group was compared to controls (i.e. no pCA or tryptophan) and a 23% increase when compared to pCA treated animals without a tryptophan load. These changes are shown in Table 18 . None of these changes were statistically significant as will be discussed in detail later.

Table 18 The percentage and direction of change for DA levels in the diencephalon for each of the four comparisons made. Controls were given pCA vehicle injection or tryptophan vehicle injection at times equivalent to treatment with these compounds. Tryptophan was administered 1 hour before death. pCA was administered either 3 days or 30 days before death. Statistical significance according to "t" tests is noted.

	3 day	30 day
control vs try	* 79% ↑	32% ↑
control vs pCA	3% ↓	2% ↑
control vs pCA + try	-	25% ↑
pCA vs pCA + try	3% ↑	23% ↑

* Statistically significant $p < .05$

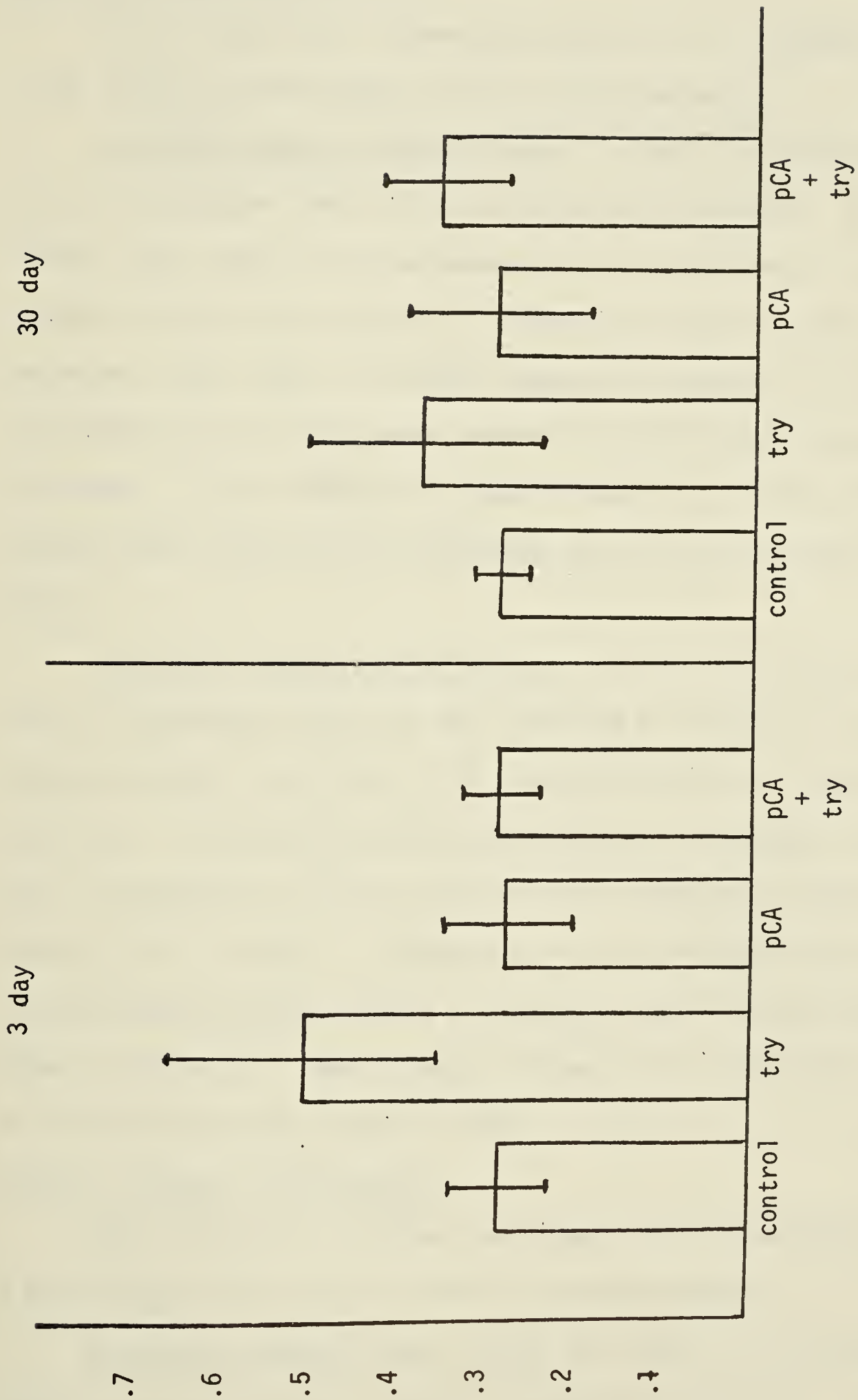


Fig. 12 Mean values of DA levels in diencephalon showing the effects of tryptophan loading (try), pCA treatment (pCA) and a combined treatment (pCA + try). One standard deviation from the mean is represented by the bars. Both 3 day and 30 day groups are shown.

(4.5.2) Changes in NA levels

Table 19 and Fig. 13 show the effects of all treatments after 3 and 30 days on the levels of NA in the diencephalon.

In animals measured after 3 days the levels of NA decreased after a tryptophan load by 6% when compared to controls. In pCA treated animals, the levels of NA decreased by 4% when compared to controls. In animals who were given pCA plus a tryptophan load, there was a 4% decrease in the levels of NA when compared to controls (i.e no pCA or tryptophan) but no change when compared to pCA treated animals without tryptophan. These changes are shown in Table 19 . None of these changes were statistically significant as will be discussed in detail later.

In animals measured after 30 days the levels of NA decreased after a tryptophan load by 6% when compared to controls. In pCA treated animals, the levels of NA increased by 4% when compared to controls. In animals who were given pCA plus a tryptophan load, there was a 2% decrease in levels of NA when this group was compared to controls (i.e. no pCA or tryptophan) and a 6% decrease when compared to pCA treated animals without a tryptophan load. These changes are shown in Table 19 . None of these changes were statistically significant as will be discussed in detail later.

(4.5.3) Changes in 5HT levels

Table 20 and Fig. 14 show the effects of all treatments after 3 and 30 days on the levels of 5HT in the diencephalon.

In animals measured after 3 days the levels of 5HT increased after a tryptophan load by 19% when compared to controls. In pCA treated animals, the levels of 5HT decreased by 50% when compared

Table 19 The percentage and direction of change for NA levels in the diencephalon for each of the four comparisons made. Controls were given pCA vehicle injections or tryptophan vehicle injections at times equivalent to treatment with these compounds. Tryptophan was administered 1 hour before death. pCA was administered either 3 days or 30 days before death. Statistical testing with "t" tests showed no significance.

	3 day	30 day
control vs try	6% ↓	6% ↓
control vs pCA	4% ↓	4% ↑
control vs pCA + try	4% ↓	2% ↓
pCA vs pCA + try	-	6% ↓

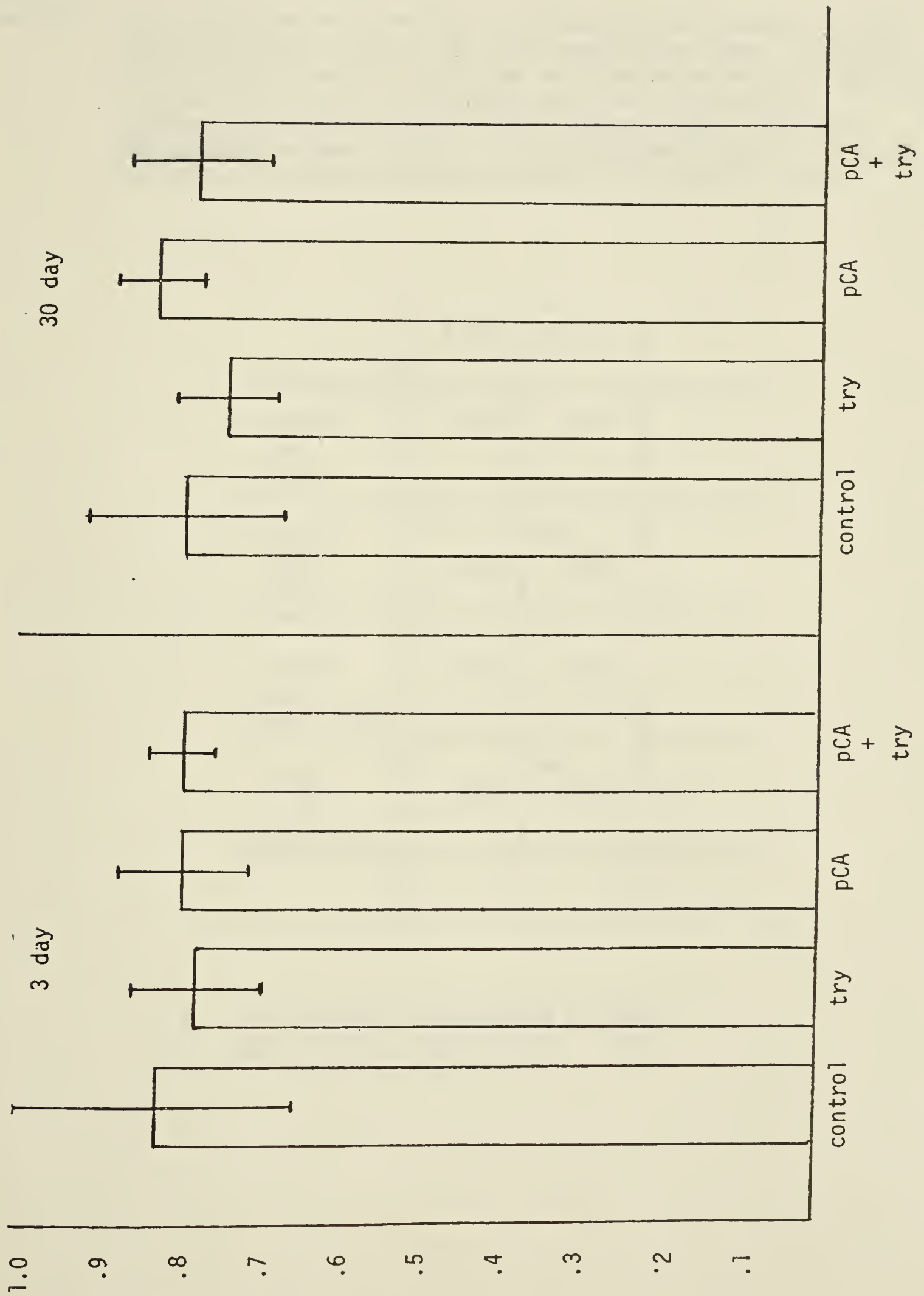


Fig. 13 Mean values of NA levels in diencephalon showing the effects of tryptophan loading (try), pCA treatment (pCA) and a combined treatment (pCA + try). One standard deviation from the mean is represented by the bars. Both 3 and 30 day groups are shown.

Table 20 The percentage and direction of change for 5HT levels in the diencephalon for each of the four comparisons made. Controls were given pCA vehicle injection or tryptophan vehicle injection at times equivalent to treatment with these compounds. Tryptophan was administered 1 hour before death. pCA was administered either 3 days or 30 days before death. Statistical significance according to "t" tests is noted.

	3 day	30 day
control vs try	19% ↑	35% ↑
control vs pCA	† 50% ↓	** 35% ↓
control vs pCA + try	24% ↓	6% ↓
pCA vs pCA + try	† 52% ↑	** 64% ↑

† statistically significant $p < .025$

** statistically significant $p < .005$

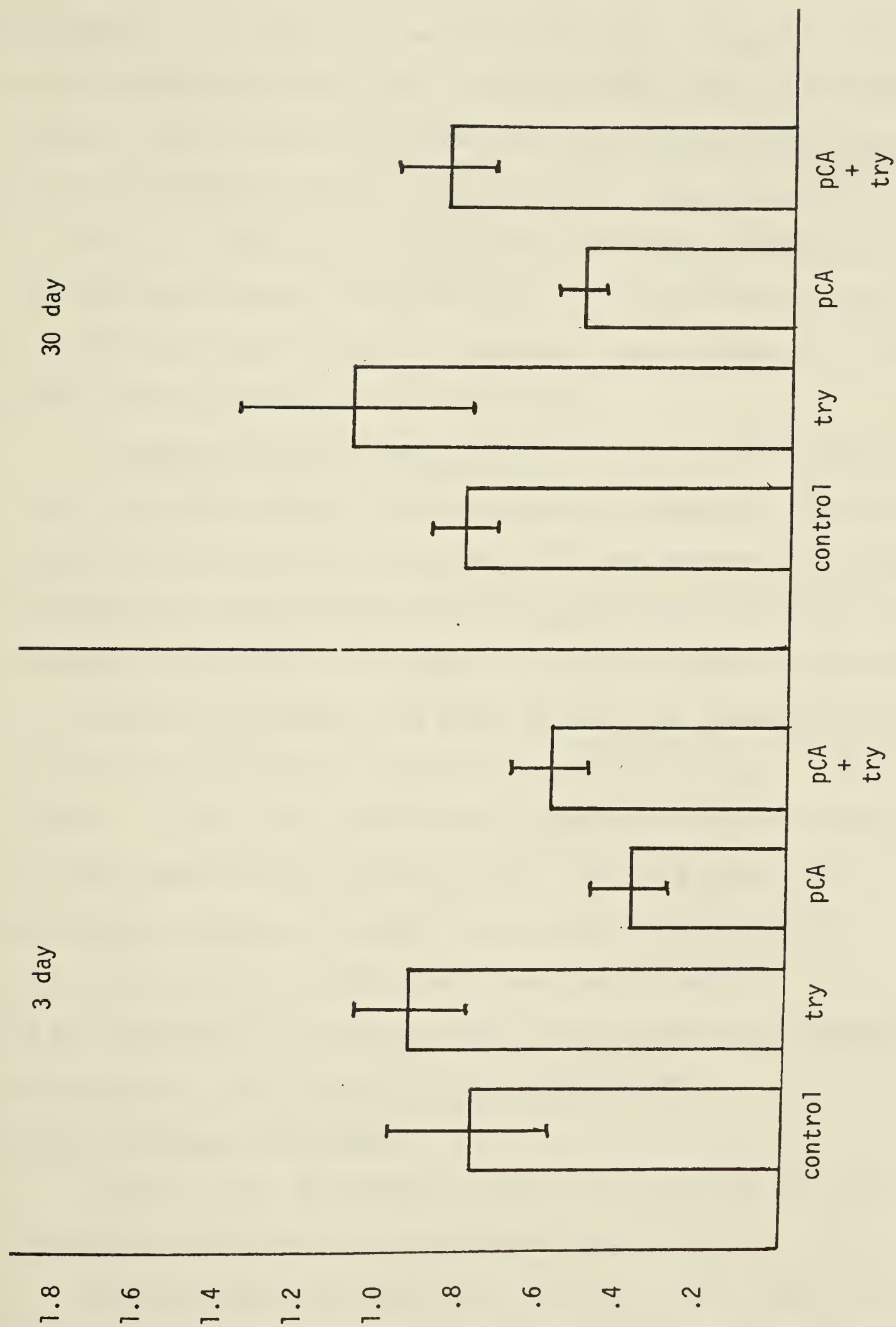


Fig. 14 Mean values of 5HT levels in diencephalon showing the effects of tryptophan loading (try), pCA treatment (pCA) and a combined treatment (pCA + try). One standard deviation from the mean is represented by the bars. Both 3 day and 30 day groups are shown.

to controls. In animals who were given pCA plus a tryptophan load there was a 24% decrease in the levels of 5HT when this group was compared to controls (i.e. no pCA or tryptophan) but a 52% increase when compared to pCA treated animals without a tryptophan load. These changes are shown in Table 20 . There was a statistically significant difference between the pCA treated animals and controls ($p < .025$) and between the pCA treated animals and the pCA plus tryptophan loaded animals ($p < .025$) which will be described in more detail later.

In animals measured after 30 days the levels of 5HT increased after a tryptophan load by 35% when compared to controls. In pCA treated animals, the levels of 5HT decreased by 35% when compared to controls. In animals who were given pCA plus a tryptophan load, there was a 6% decrease in the levels of 5HT when this group was compared to controls (i.e. no pCA or tryptophan) and a 64% increase when compared to pCA treated animals without a tryptophan load. These changes are shown in Table 20 . There was a statistically significant difference between the pCA treated animals and controls ($p < .005$) and between the pCA treated animals and the pCA plus tryptophan loaded animals ($p < .005$) which will be described in more detail later.

(4.6) The effects of different times (3 or 30 days after treatment) on the levels of DA, NA and 5HT in the diencephalon.

(4.6.1) Changes in DA levels

Table 21 shows the effects of time on the levels of DA in the diencephalon for each of the experimental groups studied.

When the levels of DA measured in control animals 3 days or 30 days after control injections were compared to one another, the levels remained constant. When the levels of DA measured in tryptophan loaded

Table 21 The percentage and direction of change for the differences in amine levels noted in the diencephalon between animals measured 3 days after treatment and animals measured 30 days after treatment. Controls were given pCA vehicle injection or tryptophan vehicle injection at times equivalent to treatment with these compounds. Tryptophan was administered 1 hour before death. pCA was administered either 3 days or 30 days before death. Statistical significance according to "t" tests is noted.

		Control	try	pCA	pCA + try
DA	3 day vs 30 day	-	26% ↓	6% ↑	26% ↑
NA	3 day vs 30 day	4% ↓	4% ↓	5% ↑	2% ↓
5HT	3 day vs 30 day	3% ↑	17% ↑	* 34% ↑	† 44% ↑

* statistically significant $p < .05$

† statistically significant $p < .025$

animals 3 days or 30 days after control injections were compared to one another, the levels were 26% lower in the group measured 30 days after injection. The levels of DA in animals treated 30 days previously with pCA were 6% higher than the levels obtained from animals measured 3 days after pCA treatment. The levels of DA in animals given a tryptophan load 30 days after pCA treatment were 26% higher than those in animals given a tryptophan load 3 days after pCA treatment. None of these differences were statistically significant as will be discussed in detail later.

(4.6.2) Changes in NA levels

Table 21 shows the effects of time on the levels of NA in the diencephalon for each of the experimental groups studied.

When the levels of NA measured in control animals 3 days or 30 days after control injections are compared to one another, the levels were 4% lower in the group measured 30 days after injection. When the levels of NA measured in tryptophan loaded animals 3 days or 30 days after control injection are compared to one another, the levels were 4% lower in the group measured 30 days after injection. The levels of NA in animals treated 30 days previously with pCA were 5% higher than the levels obtained from animals measured 3 days after pCA treatment. The levels of NA in animals given a tryptophan load 30 days after pCA treatment were 2% lower than those in animals given a tryptophan load 3 days after pCA treatment. None of these differences were statistically significant as will be discussed in detail later.

(4.6.3) Changes in 5HT levels

Table 21 shows the effects of time on the levels of 5HT in the diencephalon for each of the experimental groups studied.

When the levels of 5HT measured in control animals 3 days or 30 days after control injections are compared to one another, the levels were 3% higher in the group measured 30 days after injection. When the levels of 5HT measured in tryptophan loaded animals 3 days or 30 days after control injection are compared to one another, the levels were 17% higher in the group measured 30 days after injection. The levels of 5HT in animals treated 30 days previously with pCA were 34% higher than the levels obtained from animals measured 3 days after pCA treatment. The levels of 5HT in animals given a tryptophan load 30 days after pCA treatment were 44% higher than those in animals given a tryptophan load 3 days after pCA treatment. There was a statistically significant difference for both pCA treated groups, either with or without a tryptophan load, between those animals measured at 3 days after treatment and those measured 30 days after treatment ($p < .05$ and $p < .025$ respectively).

(4.7) Results of analysis of variance done on the amounts of individual amines in the diencephalon.

Analysis of variance of DA in the diencephalon reveals that tryptophan loading had a statistically significant effect on the levels of this amine in this area of the brain ($\alpha = .01$). Results of this statistical analysis can be seen in Table 22.

An analysis of variance done on the amounts of each of the individual amines in the diencephalon reveals that there was no

Table 22 Results of analysis of variance done for DA in the diencephalon.

Source	DF	SS	MS	F	Level of significance
pCA	1	.028655	.028655	3.657407	n.s.
try	1	.071642	.071642	9.143916	.01
pCA x try	1	.027037	.027037	3.450844	n.s.
time	1	.000755	.000755	.096396	n.s.
pCA x time	1	.022951	.022951	2.929362	n.s.
try x time	1	.002748	.002748	.350766	n.s.
pCA x try x time	1	.017086	.017086	2.180712	n.s.
Error	23	.180203	.007835		
Total	30	.351077			

Table 23 Results of analysis of variance done for NA in the diencephalon.

Source	DF	SS	MS	F	Level of significance
pCA	1	.000327	.000327	.028610	n.s.
try	1	.007096	.007096	.621650	n.s.
pCA x try	1	.002033	.002033	.178130	n.s.
time	1	.000392	.000392	.034358	n.s.
pCA x time	1	.005300	.005300	.464371	n.s.
try x time	1	.002742	.002742	.240242	n.s.
pCA x try x time	1	.002214	.002214	.193977	n.s.
Error	23	.262525	.011414		
Total	30	.282630			

Table 24 Results of analysis of variance done for 5HT in the diencephalon

Source	DF	SS	MS	F	Level of significance
pCA	1	.722556	.722556	36.240732	.005
try	1	.444136	.444136	22.276232	.005
pCA x try	1	.005712	.005712	.286473	n.s.
time	1	.160592	.160592	8.054682	.01
pCA x time	1	.021488	.021488	1.077780	n.s.
try x time	1	.032054	.032054	1.607711	n.s.
pCA x try x time	1	8.333×10^{-8}	8.333×10^{-8}	4.179693×10^{-6}	n.s.
Error	23	.458566	.019938		
Total	30	1.845104			

statistically significant difference in the effects of either pCA, tryptophan or time on NA in this area of the brain (Table 23).

Analysis of variance of 5HT in the diencephalon reveals that tryptophan loading and treatment with pCA had a statistically significant effect on the levels of this amine in this area of the brain ($\alpha = .005$ in both cases). Time also had a statistically significant effect on the levels of this amine in the diencephalon ($\alpha = .01$). Results of this analysis can be seen in Table 24 .

(4.8) Results of "t" tests done on individual groupings within the diencephalon where significance was noted after the analysis of variance.

"t" tests done on the effects of tryptophan on the levels of DA in the diencephalon are shown in Table 25 .

Table 25 Results of "t" tests for the levels of DA in animals who have been given tryptophan compared to those who have not been given tryptophan.

<u>Without</u>	<u>With</u>	<u>t</u>	<u>Level of Significance</u>	<u>Degrees of Freedom</u>
3 day	3 day	2.804	.05	6
30 day	30 day	1.367	n.s.	5
3 day	3 day pCA	.007	n.s.	6
30 day	30 day pCA	1.920	n.s.	6
3 day pCA	3 day pCA	.213	n.s.	6
30 day pCA	30 day pCA	1.051	n.s.	6

In animals who had not been given pCA the changes in DA levels seen after a tryptophan load were statistically significant 3 days but not 30 days after a control injection. None of the other changes in the levels of DA noted after a tryptophan load were statistically significant.

"t" tests done on the effects of tryptophan on the levels of 5HT in the diencephalon are shown in Table 26 .

Table 26 Results of "t" tests for the levels of 5HT in groups who have been given tryptophan compared to those who have not been given tryptophan.

Without	With	t	Level of Significance	Degrees of Freedom
3 day	3 day	1.233	n.s.	6
30 day	30 day	1.916	n.s.	5
3 day	3 day pCA	1.662	n.s.	6
30 day	30 day pCA	.698	n.s.	6
3 day pCA	3 day pCA	3.101	.025	6
30 day pCA	30 day pCA	5.015	.005	6

In animals who had not been given pCA the changes in 5HT after a tryptophan load were not statistically significant. Both 3 days and 30 days after pCA treatment the changes noted in 5HT after a tryptophan load were statistically significant when the levels are compared to animals who have been given pCA, but were not statistically significant when compared to controls (i.e. no pCA or tryptophan).

"t" tests done on the effects of pCA on the levels of 5HT in the diencephalon are shown in Table 27 .

Table 27 Results of "t" tests for the levels of 5HT in animals who who have been given pCA compared to those who have not been given pCA.

<u>Without</u>	<u>With</u>	<u>t</u>	<u>Level of Significance</u>	<u>Degrees of Freedom</u>
3 day	3 day	3.543	.025	6
30 day	30 day	5.943	.005	6
3 day try	3 day try	4.010	.01	6
30 day try	30 day try	1.462	n.s.	5

The differences in the levels of 5HT between animals who had been given pCA and those who had not were statistically significant both 3 days and 30 days after pCA treatment. In animals who had been given tryptophan along with pCA the levels of 5HT differed with statistical significance from those who had just been given tryptophan alone 3 days after treatment but were not statistically significant 30 days after treatment.

"t" tests done on the levels of 5HT at 3 and 30 days in the diencephalon are shown in Table 28 .

Table 28 Results of "t" tests for the levels of 5HT comparing animals 3 days after treatment to animals 30 days after treatment.

<u>3 day</u>	<u>30 day</u>	<u>t</u>	<u>Level of Significance</u>	<u>Degrees of Freedom</u>
Control	Control	.251	n.s.	6
Try	Try	.986	n.s.	5
pCA	pCA	2.596	.05	6
pCA plus try	pCA plus try	3.366	.025	6

With respect to the levels of 5HT in the diencephalon, there was no statistically significant difference between controls (i.e. no pCA or tryptophan) when measurements are made 3 or 30 days after a control injection. Likewise, with respect to the levels of this amine, there was no statistically significant difference between animals who had been given tryptophan 3 or 30 days after a control injection. Again with respect to the levels of 5HT in the diencephalon there was a statistically significant difference between animals who had received pCA 3 days before measurement compared to animals who had received pCA 30 days before measurement. There was also a statistically significant difference between the levels of 5HT in animals who had been given tryptophan 3 days after a pCA treatment compared to animals who had been given tryptophan 30 days after a pCA treatment.

(4.9) The effects after 3 and 30 days of tryptophan loading, pCA and pCA plus tryptophan loading on DA, NA and 5HT in the hippocampus.

(4.9.1) Changes in DA levels

Levels of DA in the hippocampus were undetectable after all treatments using the technique employed in this experiment. The quantitative level of sensitivity for DA was 60 ng/gm.

(4.9.2) Changes in NA levels

Table 29 and Fig. 15 show the effects of all treatments after 3 and 30 days on the levels of NA in the hippocampus.

In animals measured after 3 days the levels of NA increased after a tryptophan load by 16% when compared to controls. In pCA treated animals the levels of NA decreased by 8% when compared to controls. In animals who were given pCA plus a tryptophan load there was a 10% decrease in the levels of NA when this group was compared to controls (i.e. no pCA or tryptophan) but there was virtually no change when compared to pCA treated animals without a tryptophan load. These changes are shown in Table 29 . None of the changes were statistically significant as will be discussed in detail later.

In animals measured after 30 days the levels of NA decreased after a tryptophan load by 22% when compared to controls. In pCA treated animals the levels of NA decreased by 16% when compared to controls. In animals who were given pCA plus a tryptophan load, there was a 5% decrease in the levels of NA when this group was compared to controls (i.e. no pCA or tryptophan) but there was a 13% increase when compared to pCA treated animals without a tryptophan load. These changes are shown in Table 29 . None of these changes were statistically

Table 29 The percentage and direction of change for NA in the hippocampus for each of the four comparisons made. Controls were given pCA vehicle injection or tryptophan vehicle injection at times equivalent to treatment with these compounds. Tryptophan was administered 1 hour before death. pCA was administered either 3 days or 30 days before death. Statistical testing with "t" tests showed no significance.

	3 day	30 day
Control vs try.	16% ↑	22% ↓
Control vs pCA	8% ↓	16% ↓
Control vs pCA + try.	10% ↓	5% ↓
pCA vs pCA + try.	-	13% ↑

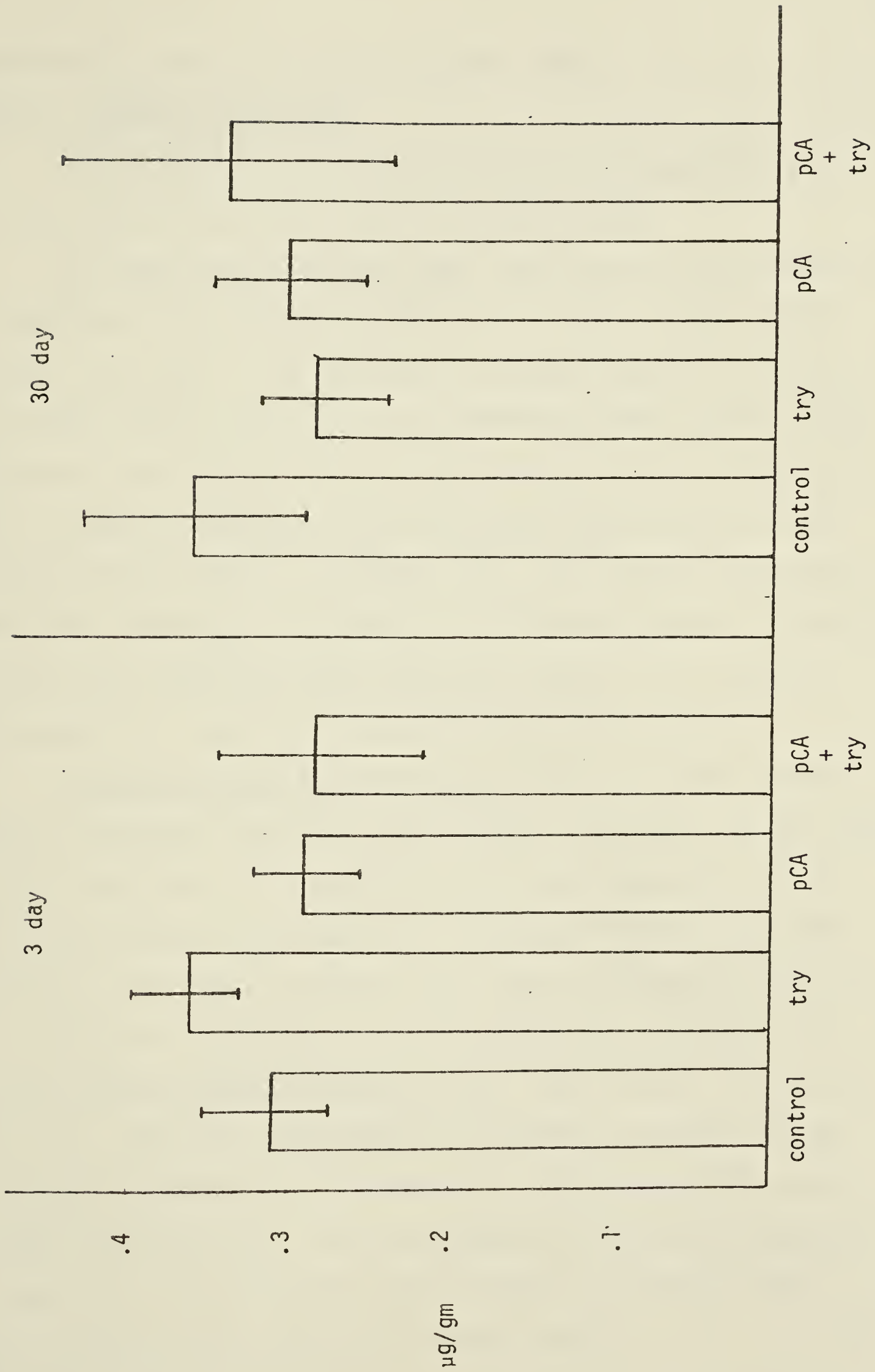


Fig. 15 Mean values of NA levels in hippocampus showing the effects of tryptophan loading (try), pCA treatment (pCA) and a combined treatment (pCA + try). One standard deviation from the mean is represented by the bars. Both 3 day and 30 day groups are shown.

significant as will be discussed in detail later.

(4.9.3) Changes in 5HT levels

Table 30 and Fig. 16 show the effects of all treatments after 3 and 30 days on the levels of 5HT in the hippocampus.

In animals measured after 3 days, the levels of 5HT increased after a tryptophan load by 59% when compared to controls. In pCA treated animals, the levels of 5HT decreased by 36% when compared to controls. In animals who were given pCA plus a tryptophan load, there was a 56% decrease in the levels of 5HT when this group was compared to controls (i.e. no pCA or tryptophan) and a 32% decrease when compared to pCA treated animals without a tryptophan load. These changes are shown in Table 30. There was a statistically significant difference between pCA treated plus tryptophan loaded animals and controls (i.e. no pCA or tryptophan) ($p < .05$) as will be discussed in detail later.

In animals measured after 30 days, the levels of 5HT increased after a tryptophan load by 81% when compared to controls. In pCA treated animals the levels of 5HT decreased by 58% when compared to controls. In animals who were given pCA plus a tryptophan load there was a 22% decrease in the levels of 5HT when this group was compared to controls (i.e. no pCA or tryptophan) but a 87% increase when compared to pCA treated animals without a tryptophan load. These changes are shown in Table 30. There was a statistically significant difference between tryptophan loaded animals and controls ($p < .01$), between pCA treated animals and controls ($p < .01$), and between pCA treated plus tryptophan loaded animals and pCA treated animals without a tryptophan load ($p < .02$) as will be discussed in detail later.

Table 30 The percentage and direction of change for 5HT levels in the hippocampus for each of the four comparisons made. Controls were given pCA vehicle injection or tryptophan vehicle injection at times equivalent to treatment with these compounds. Tryptophan was administered 1 hour before death. pCA was administered either 3 days or 30 days before death. Statistical significance according to "t" tests is noted.

	3 day	30 day
Control vs try.	59% ↑	§ 81% ↑
Control vs pCA	36% ↓	§ 58% ↓
Control vs pCA + try.	* 56% ↓	22% ↓
pCA vs pCA + try.	32% ↓	Φ 87% ↑

* Statistically significant $p < .05$

Φ Statistically significant $p < .02$

§ Statistically significant $p < .01$

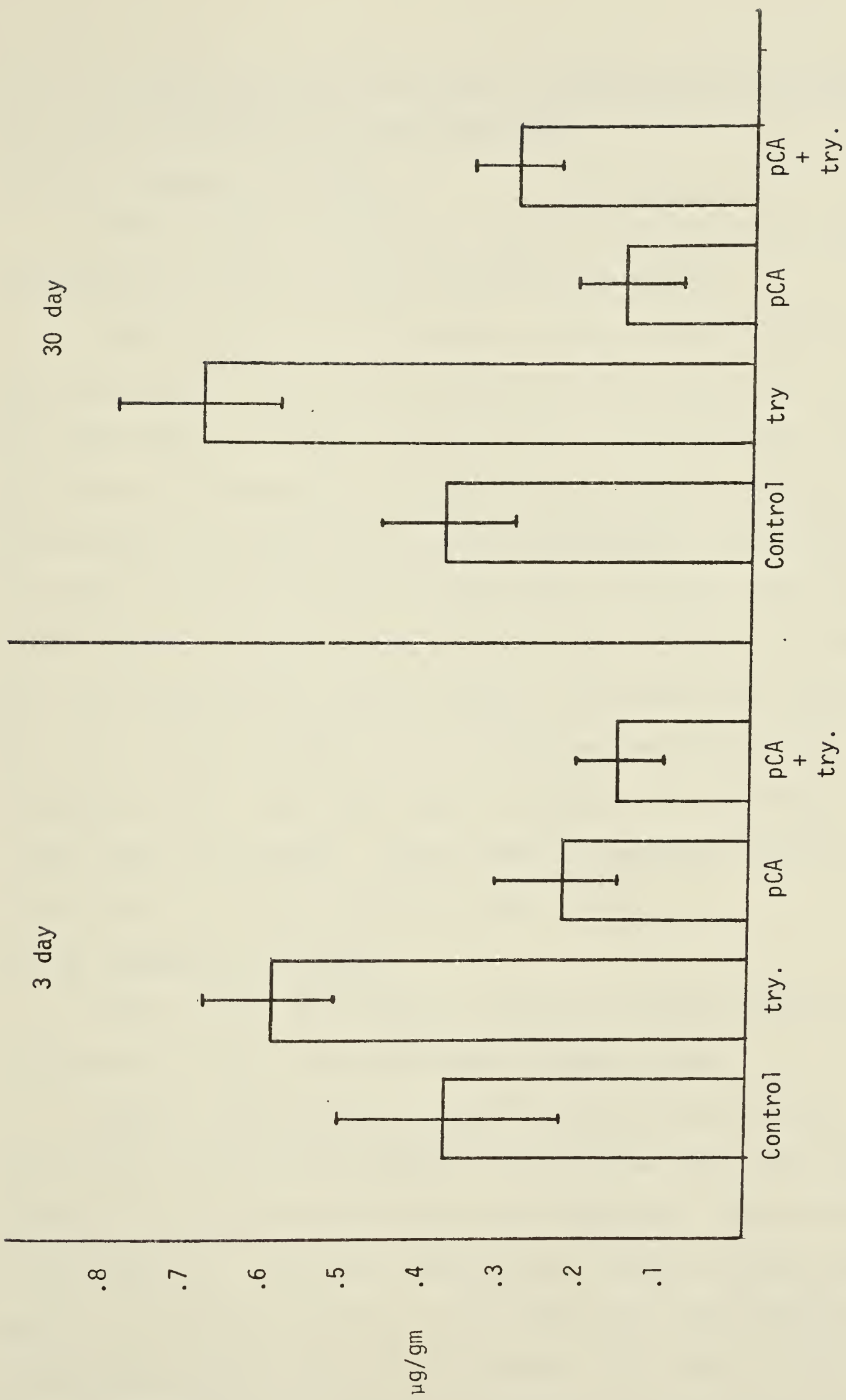


Fig. 16 Mean values of 5HT levels in hippocampus showing the effects of tryptophan loading (try), pCA treatment (pCA) and a combined treatment (pCA + try). One standard deviation from the mean is represented by the bars. Both 3 day and 30 day groups are shown.

(4.10) The effects of different times (3 or 30 days after treatment) on the levels of NA and 5HT in the hippocampus.

(4.10.1) Changes in NA levels

Table 31 shows the effects of time on the levels of NA in the hippocampus for each of the experimental groups studied.

When the levels of NA measured in control animals 3 days or 30 days after control injections are compared to one another, the levels were 16% higher in the group measured 30 days after injection. When the levels of NA measured in tryptophan loaded animals 3 days or 30 days after control injections are compared to one another, the levels were 23% lower in the group measured 30 days after injection. The levels of NA in animals treated 30 days previously with pCA were 5% higher than the levels obtained from animals measured 3 days after pCA treatment. The levels of NA in animals given a tryptophan load 30 days after pCA treatment were 22% lower than those in animals given a tryptophan load 3 days after pCA treatment. None of these differences were statistically significant as will be discussed in detail later.

(4.10.2) Changes in 5HT levels

Table 31 shows the effects of time on the levels of 5HT in the hippocampus for each of the experimental groups studied.

When the levels of 5HT measured in control animals 3 days or 30 days after control injection are compared to one another the levels were 2% higher in the group measured 30 days after injection. When the levels of 5HT measured in tryptophan loaded animals 3 days or 30 days after control injection are compared to one another, the levels were 15% higher in the group measured 30 days after injection. The levels of 5HT in animals treated 30 days previously with pCA were 33% lower than the

Table 31 The percentage and direction of change for the difference in amine levels noted in the hippocampus between animals measured 3 days after treatment and animals measured 30 days after treatment. Controls were given pCA vehicle injection or tryptophan vehicle injection at times equivalent to treatment with these compounds. Tryptophan was administered 1 hour before death. pCA was administered either 3 days or 30 days before death. Statistical significance according to "t" tests is noted.

		Control	try	pCA	pCA + try
NA	3 day vs 30 day	16% ↑	23% ↓	5% ↑	22% ↓
5HT	3 day vs 30 day	2% ↑	15% ↑	33% ↓	+ 82% ↑

+ statistically significant $p < .025$

levels obtained from animals measured 3 days after pCA treatment. The levels of 5HT in animals given a tryptophan load 30 days after pCA treatment were 82% higher than those in animals given a tryptophan load 3 days after pCA treatment. There was a statistically significant difference for pCA treated plus tryptophan loaded animals between those animals measured 3 days after treatment and those measured 30 days after treatment ($p < .025$).

(4.11) Results of analysis of variance done on the amounts of individual amines in the hippocampus.

An analysis of variance of NA in the hippocampus reveals that there was no statistically significant difference in the effects of either pCA, tryptophan or time on the levels of this amine in this area of the brain. The results of this analysis can be seen in Table 32 .

An analysis of variance of 5HT in the hippocampus reveals that both treatment with pCA and tryptophan loading had a statistically significant effect on the levels of this amine in this area of the brain ($\alpha = .005$ in both cases). The results of this analysis can be seen in Table 33 .

There was also a statistically significant pCA x tryptophan interaction ($\alpha = .005$) which indicates that changes in the levels of 5HT after a tryptophan load are dependent on whether or not the animal had been treated with pCA. Fig. 17 shows the effects of a tryptophan load in animals who had been given pCA before the tryptophan load and those who had not been given pCA before the tryptophan load. Averaged over all conditions animals who had been treated at 3 and 30 days before measurement: in those who had not been treated with pCA the levels of 5HT increased dramatically after a tryptophan load whereas in animals who had been given pCA previous to a tryptophan load the levels of 5HT increased only moderately.

Table 32 Results of analysis of variance done for NA in the hippocampus.

Source	DF	SS	MS	F	Level of significance
pCA x try x time	1	.004884	.004884	.490877	n.s.
try	1	2.777×10^{-6}	2.77×10^{-6}	.000848	n.s.
pCA x try	1	.001946	.001946	.593911	n.s.
time	1	.000887	.000887	.270651	n.s.
pCA x time	1	.005378	.005378	1.641453	n.s.
try x time	1	.003086	.003086	.942064	n.s.
pCA x try x time	1	.014082	.014082	4.298165	n.s.
Error	21	.068801	.003276		
Total	28	.099067			

Table 33 Results of analysis of variance done for 5HT in the hippocampus.

Source	DF	SS	MS	F	Level of significance
pCA	1	.635044	.635044	86.0656	.005
try	1	.160389	.160389	21.73708	.005
pCA x try	1	.098939	.098939	13.408974	.005
time	1	.011136	.011136	1.509256	n.s.
pCA x time	1	.000928	.000928	.125729	n.s.
try x time	1	.041631	.041631	5.64210	.05
pCA x try x time	1	.007464	.007464	1.011533	n.s.
Error	22	.162329	.007379		
Total	29	1.117861			

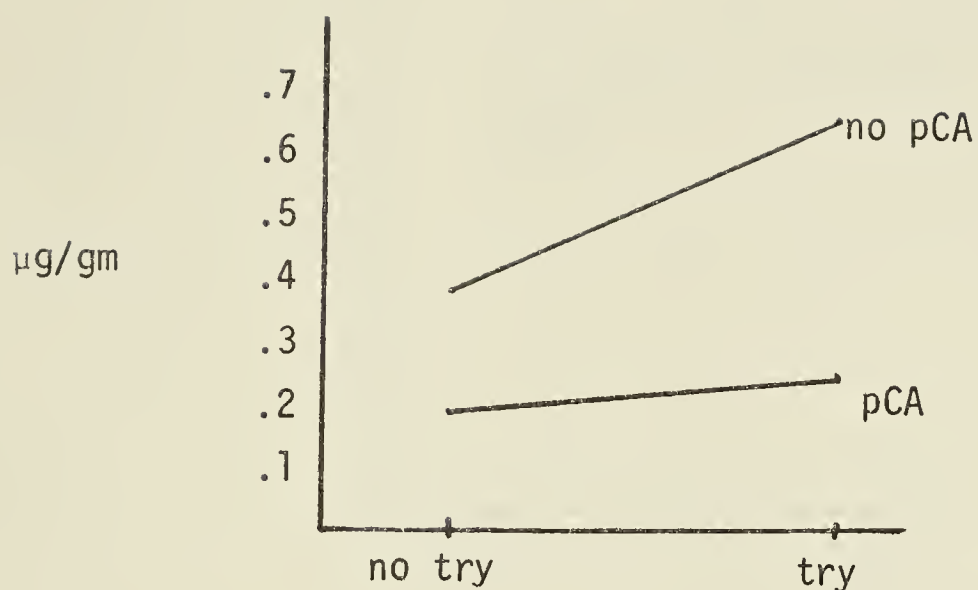


Fig. 17 The effects of tryptophan loading on the levels of 5HT in the hippocampus in animals without previous pCA treatment or in animals treated previously with pCA. Each point represents the combined means of animals treated at both 3 and 30 days before measurement.

There was also a statistically significant tryptophan x time interaction ($\alpha = .05$) which indicates that changes in 5HT after a tryptophan load are dependent on the time (3 or 30 days after pCA or control injections) at which the load was given. Fig. 18 shows the effects of a tryptophan load in animals 3 days after a pCA or control injection and in animals 30 days after a pCA or control injection. All animals were given pCA or control injections. When tryptophan was administered 30 days after the injection the animals showed a dramatic increase in the levels of 5HT but little change when the tryptophan was administered 3 days after these injections.

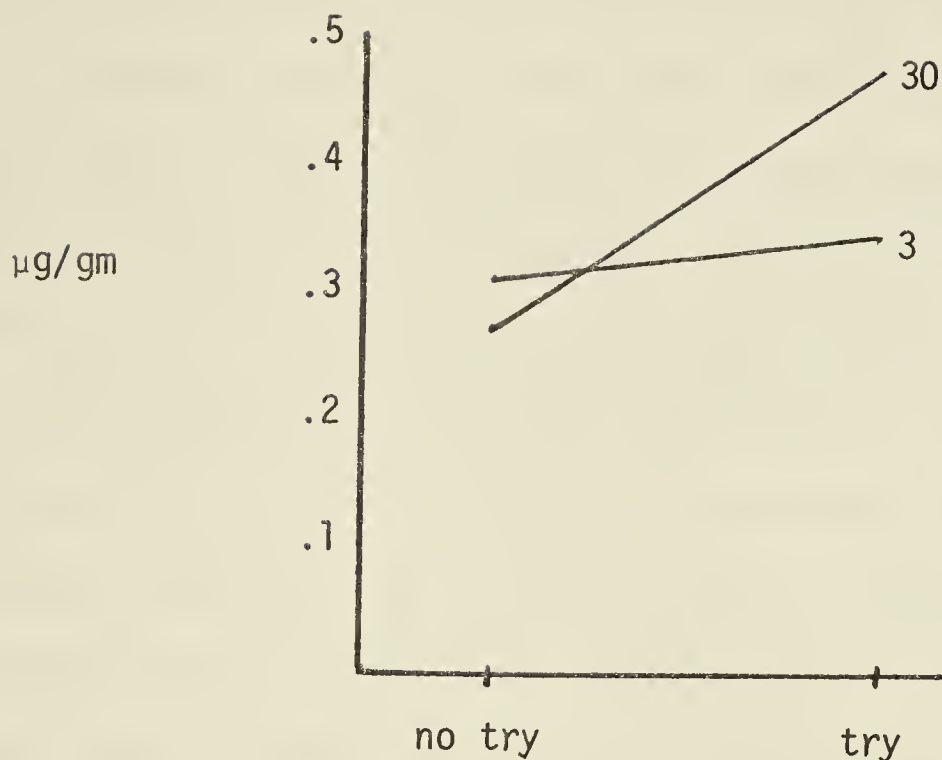


Fig. 18 The effects of tryptophan loading on the levels of 5HT in the hippocampus at both 3 days and 30 days after treatment. Each point represents the combined means of animals who have been treated with pCA and control injections.

(4.12) Results of "t" tests done on individual groupings within the hippocampus where significance was noted after the analysis of variance.

"t" tests done on the effects of tryptophan on the levels of 5HT in the hippocampus are shown in Table 34 .

Table 34 Results of "t" tests for the levels of 5HT in animals who have been given tryptophan compared to those who have not been given tryptophan.

Without	With	t	Level of Significance	Degrees of Freedom
3 day	3 day	2.388	n.s.	5
30 day	30 day	4.527	.01	5
3 day	3 day pCA	2.773	.05	6
30 day	30 day pCA	1.693	n.s.	6
3 day pCA	3 day pCA	1.541	n.s.	6
30 day pCA	30 day pCA	3.248	.02	6

In animals who had not been given pCA the changes in 5HT seen after a tryptophan load were statistically significant 30 days but not at 3 days after a control injection. In animals who had been given pCA plus a tryptophan load the changes in the levels of 5HT were statistically significant 3 days but not 30 days after treatment when compared to controls (i.e. no pCA or tryptophan). When the group of animals who had been given pCA plus a tryptophan load were compared to animals who had been given pCA alone, the changes in the levels of 5HT were statistically significant 30 days but not 3 days after treatment. "t" tests done on the effects of pCA on the levels of 5HT in the hippocampus are shown in Table 35 .

Table 35 Results of "t" tests for the levels of 5HT in animals who have been given pCA compared to those who have not been given pCA.

<u>Without</u>	<u>With</u>	<u>t</u>	<u>Level of Significance</u>	<u>Degrees of Freedom</u>
3 day	3 day	1.668	n.s.	6
30 day	30 day	4.245	.01	6
3 day try	3 day try	8.259	.001	5
30 day try	30 day try	6.711	.005	5

The differences in the levels of 5HT between animals who had been given pCA and those who had not were statistically significant when measurements are taken 30 days after treatment but were not statistically significant 3 days after treatment. In animals who had been given tryptophan along with pCA the levels of 5HT differed with statistical significance from those who had just been given tryptophan alone both 3 and 30 days after treatment.

"t" tests done on the levels of 5HT at 3 and 30 days in the hippocampus are shown in Table 36 .

Table 36 Results of "t" tests for the levels of 5HT comparing animals 3 days after treatment to animals 30 days after treatment.

<u>3 day</u>	<u>30 day</u>	<u>t</u>	<u>Level of Significance</u>	<u>Degrees of Freedom</u>
Control	Control	.084	n.s.	6
Try	Try	1.249	n.s.	4
pCA	pCA	1.436	n.s.	6
pCA + try	pCA + try	2.978	.025	6

With respect to the levels of 5HT in the hippocampus, there was no statistically significant difference between controls (i.e. no pCA or tryptophan) when measurements were made 3 or 30 days after a control injection. Likewise, with respect to the levels of this amine, there was no statistically significant difference between animals who had been given tryptophan 3 or 30 days after a control injection. Again, with respect to the levels of 5HT in the hippocampus between animals who had been given pCA 3 days before measurement compared to animals who had been given pCA 30 days before measurement there was no statistically significant difference. There was a statistically significant difference between the levels of 5HT in animals who had been given tryptophan 3 days after a pCA treatment compared to animals who had been given tryptophan 30 days after a pCA treatment.

(4.13) The effects after 3 and 30 days of tryptophan loading, pCA and pCA plus tryptophan loading on DA, NA and 5HT in the corpus striatum.

(4.13.1) Changes in DA levels

Table 37 and Fig. 19 show the effects of all treatments after 3 and 30 days on the levels of DA in the corpus striatum.

In animals measured after 3 days the levels of DA increased after a tryptophan load by 6% when compared to controls. In pCA treated animals the levels of DA decreased by 11% when compared to controls. In animals who were given pCA plus a tryptophan load, there was a 2% increase in the levels of DA when compared to controls (i.e. no pCA or tryptophan) and a 15% increase when compared to pCA treated animals without a tryptophan load. These changes are shown in Table 37 . None of these changes were statistically significant as will be discussed in detail later.

In animals measured after 30 days the levels of DA did not change after a tryptophan load when compared to controls. In pCA treated animals the levels of DA increased by 2% when compared to controls. In animals who were given pCA plus a tryptophan load, there was a 3% increase in the levels of DA when this group was compared to controls, (i.e. no pCA or tryptophan) but there was no change when compared to pCA treated animals without a tryptophan load. These changes are shown in Table 37 . None of these changes were statistically significant as will be discussed in detail later.

(4.13.2) Changes in NA levels

Table 38 and Fig. 20 show the effects of all treatments after 3 and 30 days on the levels of NA in the corpus striatum.

Table 37 The percentage and direction of change for DA levels in the corpus striatum for each of the four comparisons made. Controls were given pCA vehicle injection or tryptophan vehicle injection at times equivalent to treatment with these compounds. Tryptophan was administered 1 hour before death. pCA was administered either 3 days or 30 days before death. Statistical testing with "t" tests showed no significance.

	3 day	30 day
Control vs try	6% ↑	-
Control vs pCA	11% ↑	2% ↑
Control vs pCA + try	2% ↑	3% ↑
pCA vs pCA + try	15% ↑	-

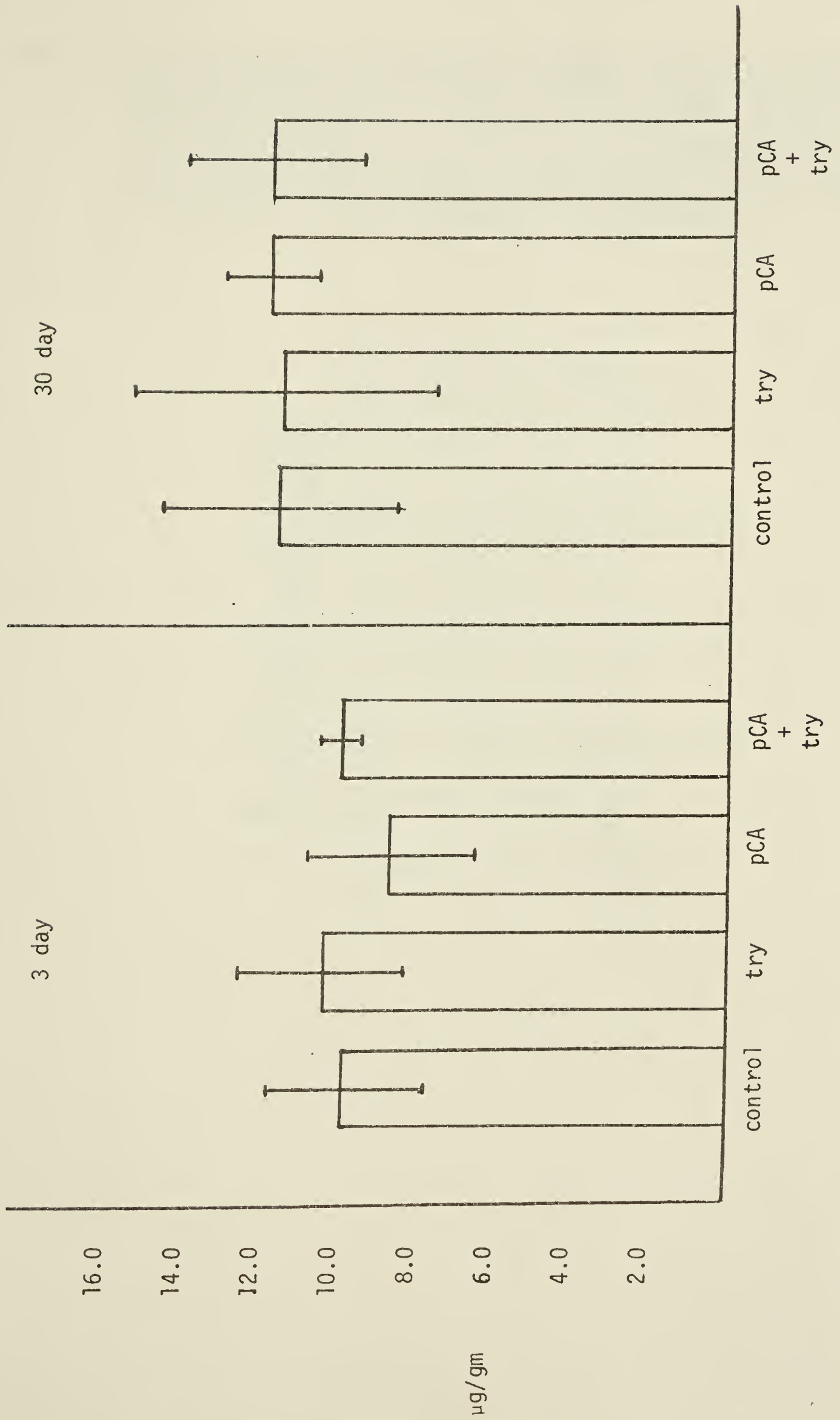


Fig. 19 Mean values of DA in corpus striatum showing the effects of tryptophan loading (try), pCA treatment (pCA) and a combined treatment (pCA + try). One standard deviation from the mean is represented by the bars. Both 3 and 30 day groups are shown.

Table 38 The percentage and direction of change for NA in the corpus striatum for each of the four comparisons made. Controls were given pCA vehicle injection or tryptophan vehicle injection, at times equivalent to treatment with these compounds. Tryptophan was administered 1 hour before death. pCA was administered either 3 days or 30 days before death. Statistical testing with "t" tests showed no significance.

	3 day	30 day
Control vs try	56% ↑	18% ↓
Control vs pCA	5% ↑	2% ↓
Control vs pCA + try	8% ↑	12% ↑
pCA vs pCA + try	2% ↑	14% ↑

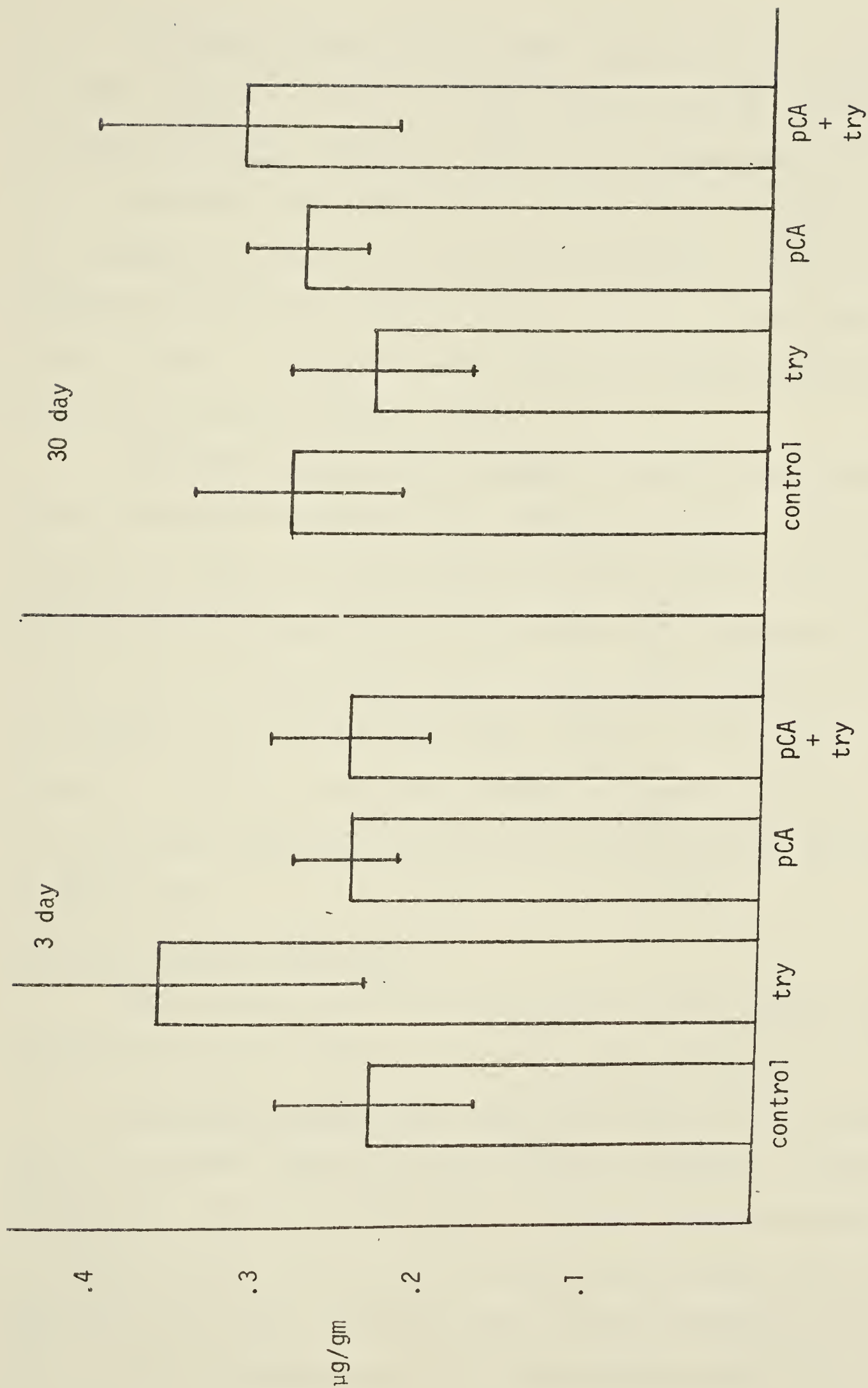


Fig. 20 Mean values of NA in corpus striatum showing the effects of tryptophan loading (try), pCA treatment (pCA) and a combined treatment (pCA + try). One standard deviation from the mean is represented by the bars. Both 3 and 30 day groups are shown.

In animals measured after 3 days the levels of NA increased by 56% when compared to controls. In pCA treated animals, the levels of NA increased by 5% when compared to controls. In animals who were given pCA plus a tryptophan load, there was an 8% increase in the levels of NA when compared to controls (i.e. no pCA or tryptophan) and a 2% increase when compared to pCA treated animals without tryptophan. These changes are shown in Table 38 . None of these changes were statistically significant as will be discussed in detail later.

In animals measured after 30 days the levels of NA decreased after a tryptophan load by 18% when compared to controls. In pCA treated animals, the levels of NA decreased by 2% when compared to controls. In animals who were given pCA plus a tryptophan load, there was a 12% increase in the levels of NA when this group was compared to controls (i.e. no pCA or tryptophan) and a 14% increase when compared to pCA treated animals without a tryptophan load. These changes are shown in Table 38 . None of these changes were statistically significant as will be discussed in detail later.

(4.13.3) Changes in 5HT levels

Table 39 and Fig. 21 show the effects of all treatments after 3 and 30 days on the levels of 5HT in the corpus striatum

In animals measured after 3 days the levels of 5HT increased after a tryptophan load by 42% when compared to controls. In pCA treated animals, the levels of 5HT decreased by 15% when compared to controls. In animals who were given pCA plus a tryptophan load there was a 13% decrease in the levels of 5HT when this group was compared to controls (i.e. no pCA or tryptophan) but a 2% increase when compared to pCA treated animals without a tryptophan load. These changes are shown

Table 39 The percentage and direction of change for 5HT in the corpus striatum for each of the four comparisons made. Controls were given pCA vehicle injections or tryptophan vehicle injections at times equivalent to treatment with these compounds. Tryptophan was administered 1 hour before death. pCA was administered either 3 days or 30 days before death. Statistical significance according to "t" tests is noted.

	3 day	30 day
Control vs try	Φ 42% ↑	69% ↑
Control vs pCA	15% ↓	30% ↓
Control vs pCA + try	13% ↓	36% ↑
pCA vs pCA + try	2% ↑	\S 93% ↑

Φ Statistically significant $p < .02$

\S Statistically significant $p < .01$

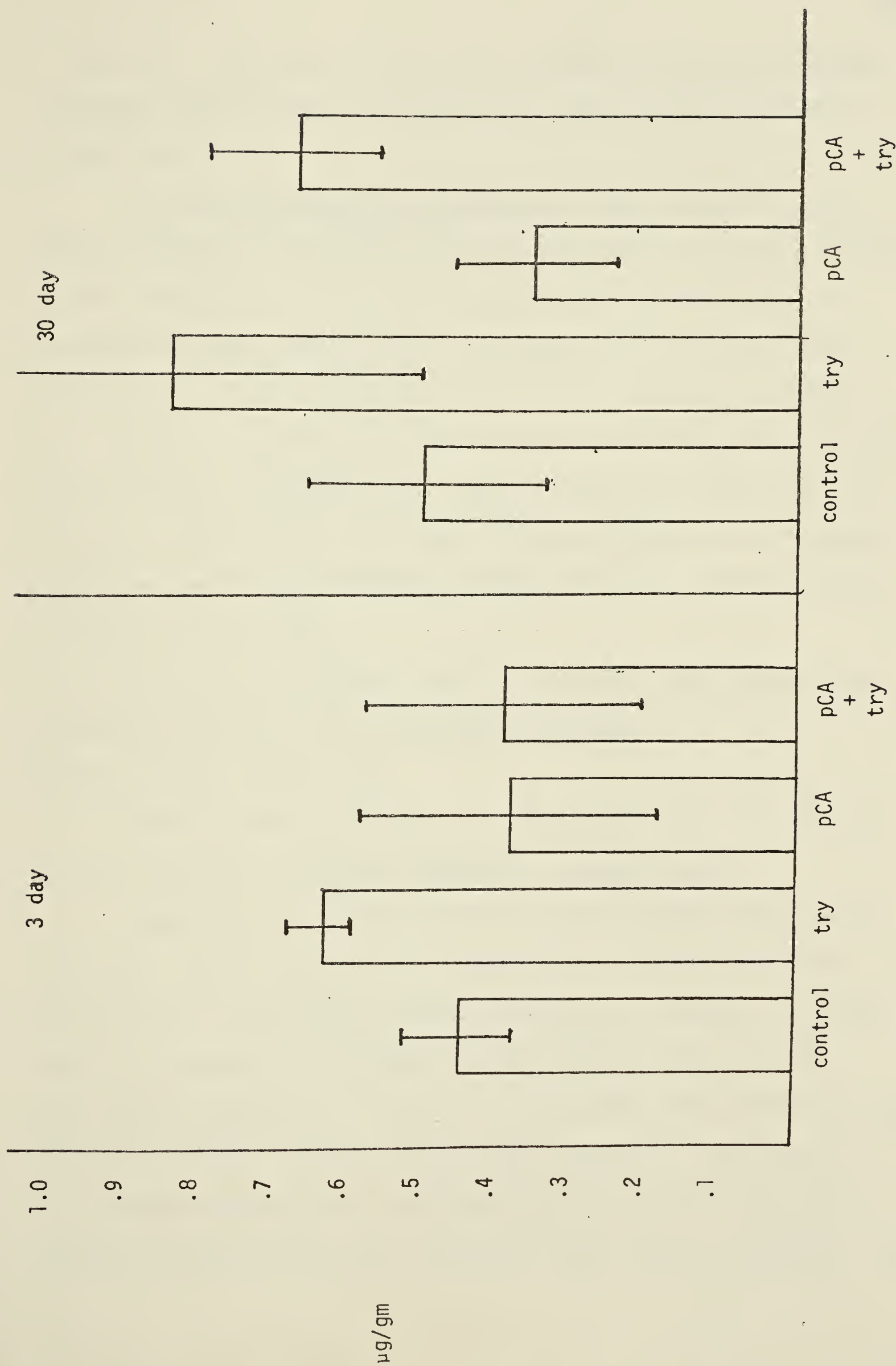


Fig. 21 Mean values of 5HT in corpus striatum showing the effects of tryptophan loading (try), pCA treatment (pCA) and a combined treatment (pCA + try). One standard deviation from the mean is represented by the bars. Both 3 and 30 day groups are shown.

in Table 39 . There was a statistically significant difference between tryptophan loaded animals and controls ($p < .02$) as will be discussed in detail later.

In animals measured after 30 days the levels of 5HT increased after a tryptophan load by 69% when compared to controls. In pCA treated animals, the levels of 5HT decreased by 30% when compared to controls. In animals who were given pCA plus a tryptophan load, there was a 36% increase in the levels of 5HT when this group was compared to controls (i.e. no pCA or tryptophan) and a 93% increase when compared to pCA treated animals without a tryptophan load. These changes are shown in Table 39 . There was a statistically significant difference between the pCA treated animals and the pCA plus tryptophan loaded animals ($p < .01$) as will be discussed in detail later.

(4.14) The effects of different times (3 or 30 days after treatment) on the levels of DA, NA and 5HT in the corpus striatum.

(4.14.1) Changes in DA levels

Table 40 shows the effects of times on the levels of DA in the corpus striatum for each of the experimental groups studied.

When the levels of DA measured in control animals 3 days or 30 days after control injections are compared to one another, the levels were 18% higher in the group measured 30 days after injection. When the levels of DA measured in tryptophan loaded animals 3 days or 30 days after control injections are compared to one another, the levels were 11% higher in the group measured 30 days after injection. The levels of DA in animals treated 30 days previously with pCA were 36% higher than the levels obtained from animals measured 3 days after pCA treatment. The

Table 40 The percentage and direction of change for the differences in amine levels noted in the corpus striatum between animals measured 3 days after treatment and animals measured 30 days after treatment. Controls were given pCA vehicle injection or tryptophan vehicle injection at times equivalent to treatment with these compounds. Tryptophan was administered 1 hour before death. pCA was administered either 3 days or 30 days before death. Statistical significance according to "t" tests is noted.

		Control	try	pCA	pCA + try
DA	3 day vs 30 day	18% ↑	11% ↑	* 36% ↑	19% ↑
NA	3 day vs 30 day	22% ↓	35% ↓	14% ↑	27% ↓
5HT	3 day vs 30 day	11% ↑	33% ↑	6% ↓	* 74% ↑

* statistically significant $p < .05$

levels of DA in animals given a tryptophan load 30 days after pCA treatment were 19% higher than those in animals given a tryptophan load 3 days after pCA treatment. There was a statistically significant difference in animals treated with pCA ($p < .05$).

(4.14.2) Changes in NA levels

Table 40 shows the effects of time on the levels of NA in the corpus striatum for each of the experimental groups studied.

When the levels of NA measured in control animals 3 days or 30 days after control injections are compared to one another the levels were 22% lower in the group measured 30 days after injection. When the levels of NA measured in tryptophan loaded animals 3 days or 30 days after control injection are compared to one another, the levels were 35% lower in the group measured 30 days after injection. The level of NA in animals treated 30 days previously with pCA were 14% higher than the levels obtained from animals measured 3 days after pCA treatment. The levels of NA in animals given a tryptophan load 30 days after pCA treatment were 27% lower than those in animals given a tryptophan load 3 days after pCA treatment. None of these differences were statistically significant as will be discussed in detail later.

(4.14.3) Changes in 5HT levels

Table 40 shows the effects of time on the levels of 5HT in the corpus striatum for each of the experimental groups studied.

When the levels of 5HT measured in control animals 3 days or 30 days after control injections are compared to one another, the levels were 11% higher in the group measured 30 days after injection. When the levels of 5HT measured in tryptophan loaded animals 3 days or 30 days after control injections are compared to one another, the levels

were 33% higher in the group measured 30 days after injection. The levels of 5HT in animals treated 30 days previously with pCA were 6% lower than the levels obtained from animals measured 3 days after pCA treatment. The levels of 5HT in animals given a tryptophan load 30 days after pCA treatment were 74% higher than those in animals given a tryptophan load 3 days after pCA treatment. There was a statistically significant time difference in animals treated with pCA plus a tryptophan load ($p < .05$).

(4.15) Results of analysis of variance done on the amounts of individual amines in the corpus striatum.

Analysis of variance of DA in the corpus striatum reveals that time has a statistically significant effect on the levels of this amine in this area of the brain ($\alpha = .05$). Results of this statistical analysis can be seen in Table 41 .

Analysis of variance of NA in the corpus striatum reveals that there is a statistically significant pCA x tryptophan x time interaction ($\alpha = .05$). Thus the levels of NA differ after a tryptophan load depending on the other two variables. Fig. 22 shows this interaction. In animals who had not been given pCA the effect of tryptophan 3 days after a control injection was to increase the levels of NA whereas 30 days after a control injection the effect of a tryptophan load decreased the levels of this amine. In animals who had been treated with pCA, a tryptophan load 3 days after pCA treatment did not effect the levels of NA, whereas 30 days after treatment a tryptophan load resulted in an increase in NA levels. Results of this statistical analysis can be seen in Table 42 .

Table 41 Results of analysis of variance done for DA in the corpus striatum.

Source	DF	SS	MS	F	Level of Significance
pCA	1	.244674	.244674	.0523	n.s.
try	1	1.413237	1.413237	.3022	n.s.
pCA x try	1	.314580	.314580	.0673	n.s.
time	1	26.862946	26.862946	5.7451	.05
pCA x time	1	1.971102	1.971102	.4215	n.s.
try x time	1	1.499072	1.499072	.3206	n.s.
pCA x try x time	1	.164256	.164256	.0351	n.s.
Error	20	93.516480	4.675820		
Total	27	125.986350			

Table 42 Results of analysis of variance done for NA in the corpus striatum.

Source	DF	SS	MS	F	Level of Significance
pCA	1	.001350	.001350	.0321	n.s.
try	1	.006353	.006353	1.5103	n.s.
pCA x try	1	.000450	.000450	.1071	n.s.
time	1	.000245	.000245	.0583	n.s.
pCA x time	1	.013950	.013950	3.3164	n.s.
try x time	1	.009440	.009440	2.2443	n.s.
pCA x try x time	1	.019581	.018581	4.6551	.05
Error	20	.084127	.004206		
Total	27	.134281			

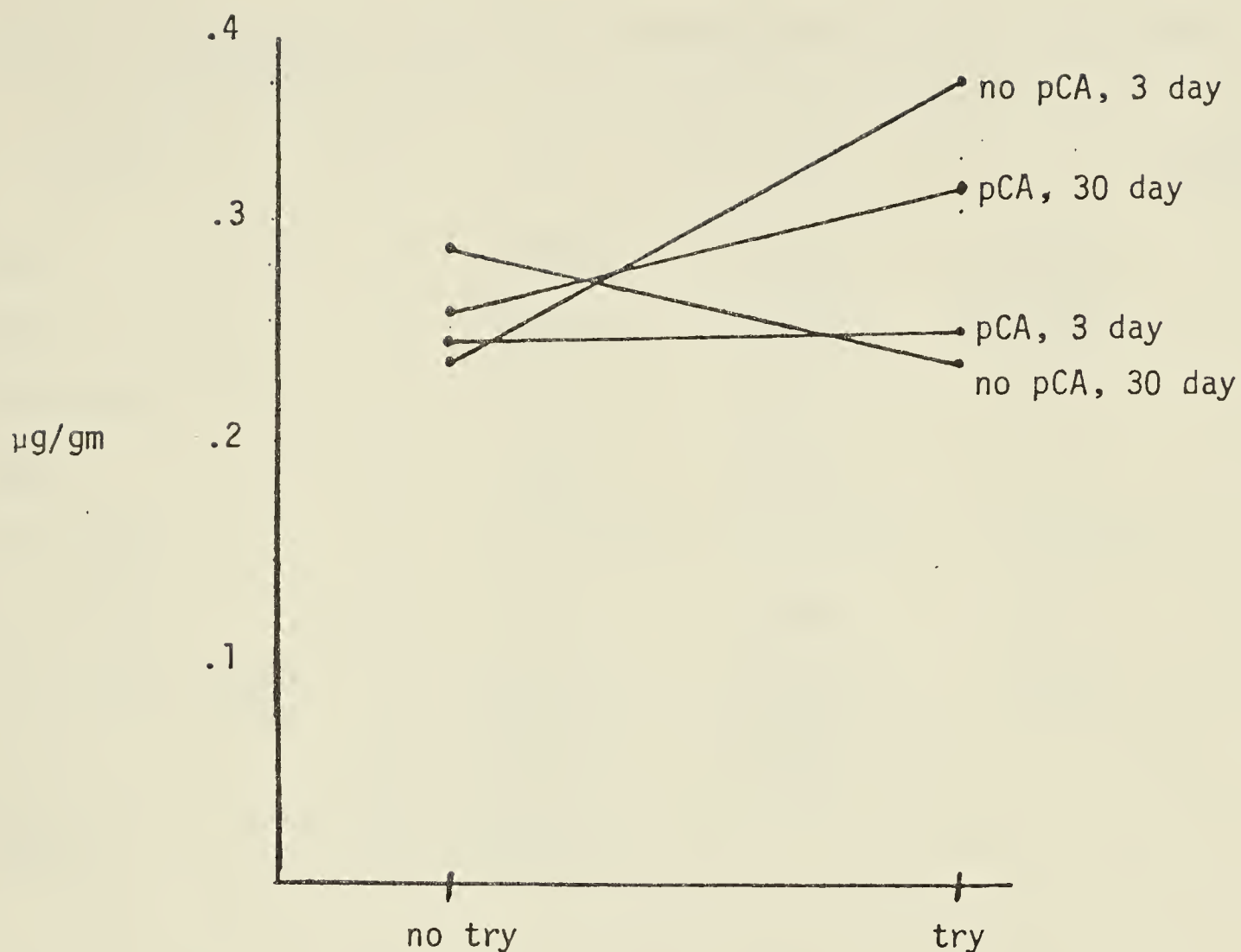


Fig. 22 The effects of a tryptophan load on the levels of NA in each of the experimental conditions

Analysis of variance of 5HT in the corpus striatum reveals that tryptophan loading and treatment with pCA have statistically significant effects on the levels of this amine in this area of the brain ($\alpha = .005$ and $.025$ respectively). See Table 43.

(4.16) Results of "t" tests done on individual groupings within the corpus striatum where significance was noted after the analysis of variance.

"t" tests done on the levels of DA at 3 and 30 days in the corpus striatum are shown in Table 44 .

Table 43 Results of analysis of variance done for 5HT in the corpus striatum.

Source	DF	SS	MS	F	Level of Significance
pCA	1	.174360	.174360	6.1185	.025
try	1	.335500	.335500	11.7798	.005
pCA x try	1	.017132	.017132	.6015	n.s.
time	1	.120795	.120795	4.2413	n.s.
pCA x time	1	1.2346×10^{-8}	1.2346×10^{-8}	4.3348×10^{-7}	n.s.
try x time	1	.101336	.101336	3.5581	n.s.
pCA x try x time	1	.011378	.011378	.3995	n.s.
Error	21	.598095	.028481		
Total	28	1.358494			

Table 44 Results of "t" tests for the levels of DA comparing animals 3 days after treatment to animals 30 days after treatment.

<u>3 day</u>	<u>30 day</u>	<u>t</u>	<u>Level of Significance</u>	<u>Degrees of Freedom</u>
Control	Control	.950	n.s.	5
Try	Try	.455	n.s.	4
pCA	pCA	2.591	.05	6
pCA + try	pCA + try	1.782	n.s.	5

With respect to the levels of DA in the corpus striatum there was no statistically significant difference between controls (i.e. no pCA or tryptophan) when measurements are made 3 or 30 days after a control injection. Likewise, with respect to the levels of this amine, there was no statistically significant difference between animals who had been given tryptophan 3 or 30 days after a control injection. Again, with respect to the levels of DA in the corpus striatum there was a statistically significant difference between animals who had been given pCA 3 days before measurement compared to animals who had been given pCA 30 days before measurement. There was no statistically significant difference between the levels of DA in animals who had been given tryptophan 3 days after a pCA treatment compared to animals who had been given tryptophan 30 days after a pCA treatment.

Since there was a statistically significant triple interaction on the levels of NA in the corpus striatum "t" tests were done on all the experimental groups for this amine. "t" tests done on the effects of tryptophan on the levels of NA in the corpus striatum are shown in Table 45.

Table 45 Results of "t" tests for the levels of NA in groups who have been given tryptophan compared to those who have not been given tryptophan.

<u>Without</u>	<u>With</u>	<u>t</u>	<u>Level of Significance</u>	<u>Degrees of Freedom</u>
3 day	3 day	1.665	n.s.	5
30 day	30 day	1.163	n.s.	5
3 day	3 day pCA	.403	n.s.	6
30 day	30 day pCA	.538	n.s.	5
3 day pCA	3 day pCA	.190	n.s.	6
30 day pCA	30 day pCA	.712	n.s.	5

None of the changes in NA levels in animals after a tryptophan load were statistically significant according to "t" tests. "t" tests done on the effects of pCA on the levels of 5HT in the corpus striatum are shown in Table 46 .

Table 46 Results of "t" tests for the levels of NA in groups who have been given pCA compared to those who have not been given pCA.

<u>Without</u>	<u>With</u>	<u>t</u>	<u>Level of Significance</u>	<u>Degrees of Freedom</u>
3 day	3 day	.380	n.s.	6
30 day	30 day	.158	n.s.	6
3 day try	3 day try	1.432	n.s.	5
30 day try	30 day try	1.361	n.s.	4

None of the changes in NA levels in animals after pCA treatment were statistically significant according to "t" tests. "t" tests done on the levels of NA at 3 and 30 days in the corpus striatum are shown in Table 47 .

Table 47 Results of "t" tests for the levels of NA comparing animals 3 days after treatment to animals 30 days after treatment.

<u>3 day</u>	<u>30 day</u>	<u>t</u>	<u>Level of Significance</u>	<u>Degrees of Freedom</u>
Control	Control	1.157	n.s.	6
Try	Try	1.648	n.s.	4
pCA	pCA	.035	n.s.	6
pCA + try	pCA + try	.691	n.s.	5

None of the changes in NA levels in animals measured 3 days after treatment compared to those measured 30 days after treatment were statistically significant according to "t" tests.

"t" tests done on the effects of tryptophan on the levels of 5HT in the corpus striatum are shown in Table 48 .

Table 48 Results of "t" tests for the levels of 5HT in groups who have been given tryptophan compared to those who have not been given tryptophan.

<u>Without</u>	<u>With</u>	<u>t</u>	<u>Level of Significance</u>	<u>Degrees of Freedom</u>
3 day	3 day	3.910	.02	5
30 day	30 day	1.580	n.s.	4
3 day	3 day pCA	.587	n.s.	6
30 day	30 day pCA	1.772	n.s.	6
3 day pCA	3 day pCA	.065	n.s.	6
30 day pCA	30 day pCA	4.235	.01	6

In animals who had not been given pCA the changes in 5HT after a tryptophan load were statistically significant when tryptophan was administered 3 days after but not 30 days after a control injection. In animals who had been given tryptophan along with pCA the levels of 5HT differed with statistical significance in animals who had been given

the tryptophan load 30 days after pCA treatment compared to those who had just received pCA alone. None of the other changes in the levels of 5HT noted after a tryptophan load were statistically significant. "t" tests done on the effects of pCA on the levels of 5HT in the corpus striatum are shown in Table 49 .

Table 49 Results of "t" tests for the levels of 5HT in animals who have been given pCA compared to those who have not been given pCA.

<u>Without</u>	<u>With</u>	<u>t</u>	<u>Level of Significance</u>	<u>Degrees of Freedom</u>
3 day	3 day	.642	n.s.	6
30 day	30 day	1.508	n.s.	5
3 day try	3 day try	2.204	n.s.	5
30 day try	30 day try	.921	n.s.	5

None of the changes in 5HT levels in animals after pCA treatment were statistically significant according to "t" tests.

Even though the analysis of variance did not show a statistically significant time variable for 5HT, the difference noted between animals who were given tryptophan 3 or 30 days after pCA treatment (74%) suggested that "t" tests should be carried out on these comparisons. "t" tests done on the levels of 5HT at 3 and 30 days in the corpus striatum are shown in Table 50 .

Table 50 Results of "t" tests for the levels of 5HT comparing animals 3 days after treatment to animals 30 days after treatment.

<u>3 day</u>	<u>30 day</u>	<u>t</u>	<u>Level of Significance</u>	<u>Degrees of Freedom</u>
Control	Control	.580	n.s.	6
Try	Try	1.047	n.s.	4
pCA	pCA	.254	n.s.	6
pCA + try	pCA + try	2.635	.05	6

With respect to the levels of 5HT in the corpus striatum there was no statistically significant difference between controls (i.e. no pCA or tryptophan) when measurements are made 3 or 30 days after a control injection. Likewise, with respect to the levels of this amine, there was no statistically significant difference between animals who had been given tryptophan 3 or 30 days after a control injection. Again with respect to the levels of 5HT in the corpus striatum, there was no statistically significant difference between animals who had been given pCA 3 days before measurement compared to animals who had been given pCA 30 days before measurement. There was a statistically significant difference between the levels of 5HT in animals who had been given tryptophan 3 days after a pCA treatment compared to animals who had been given tryptophan 30 days after a pCA treatment.

(5.) DISCUSSION

The importance of conducting a regional analysis of the brain to determine the effects of pCA on the levels of DA, NA and 5HT is shown by the results of this experiment. Some of the areas chosen for this study have not been analyzed as such in the literature. The "hippocampus" in this experiment includes the dentate gyrus. Whether or not this structure is included by other authors in their work on this area is, in some instances, unknown. The corpus striatum in this experiment includes the lenticular nucleus (putamen and globus pallidus) and the caudate nucleus. Terminology of the basal ganglia needs some clarification. The striatum which actually consists of the putamen and the caudate and excludes the globus pallidus is sometimes used in the literature as meaning the corpus striatum. The "diencephalon" in this experiment refers to all areas of the thalamus and the hypothalamus. In most other work these two areas are analyzed separately. The mesencephalon-pons analyzed in this experiment is unique in that other authors usually include the mesencephalon with midbrain dissections and the pons with the medulla oblongata. It seemed that to meet the objectives outlined for this experiment of studying a cell body area, compared to terminal areas, dissections as performed were logical. Because of such differences in dissections and the use of imprecise terminology, when comparing the results obtained in this experiment to results obtained by others, except in the case of the corpus striatum and possibly the hippocampus, caution must prevail.

Where comparable, the control values obtained for the various areas reported in this experiment are consistent with the literature

with the one exception of DA in the corpus striatum. The values found here for DA in the corpus striatum ($10.46 \pm 2.4 \mu\text{g/gm}$; combined 3 day and 30 day values) are higher than those reported in the literature ($3.55 \pm 0.4 \mu\text{g/gm}$ - $7.11 \pm 1.62 \mu\text{g/gm}$; see Table 1). These differences are due most likely to differences in extraction procedures and analytical techniques. Recently Boulton (1976) reported that DA levels in the caudate nucleus (which according to descriptions from his earlier papers is identical to the corpus striatum) were in excess of $12 \mu\text{g/gm}$. These levels were obtained using mass spectrometry, suggesting that other values reported in the literature may be low.

The results from this experiment show that the effect of a tryptophan load at 100 mg/kg 1 hour before death varies according to the area studied. In animals which were injected with tryptophan 3 days after a pCA vehicle treatment there was a statistically significant increase in the levels of DA in the diencephalon and a statistically significant increase in the levels of 5HT in the corpus striatum. In animals injected with tryptophan 30 days after a pCA vehicle treatment there was a statistically significant increase in the levels of 5HT in the hippocampus. There was little or no change in the levels of NA.

It seems logical that a tryptophan load should not affect levels of NA or DA. The change noted in the levels of DA in the diencephalon is therefore confusing. Barbeau (1972) has suggested that changes in 5HT synthesis may be regulated by DA. Alternatively, a tryptophan load may indirectly increase the levels of DA through physiological processes such as changes in the autonomic nervous system (since both 5HT and DA may be involved in regulating this system) or hormonal feedback in the median eminence (Barbeau, 1972; Donovan, 1970). Since

there is no evidence for any of the above suggestions the observed increase in DA remains unexplained and should be considered suspect until either the finding is replicated or one of the alternative explanations is proven.

The increases in 5HT in the hippocampus and the corpus striatum found in this study are consistent with the work of Curzon and Marsden (1975) and Knott and Curzon (1974). They found a significant increase in 5HT levels after a tryptophan load only in the midbrain and hippocampus, while 5HIAA increased in the midbrain and hippocampus and the corpus striatum.

In all areas studied, pCA treatment had little effect on the levels of NA and DA regardless of whether measurements were made 3 days or 30 days after pCA treatment but had a dramatic effect on 5HT levels after both time intervals. The changes in levels of the catecholamines after pCA treatment reported by previous workers (Miller et al., 1970; Morgan et al., 1972; Leonard, 1976) were not found here. It may be assumed, therefore, that these reported changes were only short term. The results of this experiment thus, support the original contention (Pletscher et al., 1964; Fuller and Hines, 1965) that pCA has a specific effect on 5HT.

pCA treatment resulted in a decrease in the levels of 5HT in all areas studied. Three days after pCA treatment the levels in the mesencephalon-pons and diencephalon were reduced by 47% and 50% respectively. Thirty days after pCA treatment, the levels of 5HT recovered slightly, but remained reduced by 40% and 35% in the two areas respectively. The reductions noted in these areas were

statistically significant at both 3 and 30 days after treatment. In the hippocampus the level of 5HT was reduced by 36% 3 days after pCA treatment whilst in the corpus striatum the level was decreased by 15% 30 days after treatment, the level in the hippocampus was decreased by 58% while that in the corpus striatum was decreased by 30%. The only statistically significant decrease in these areas was that noted in the hippocampus 30 days after treatment.

The reduction of 5HT in the diencephalon agrees with the findings reported after 24 hours by Costa et al. (1971). Similar changes have been reported for the hypothalamus by Morgan et al. (1972) and Sanders-Bush et al. (1975) after 4 hours and by Sanders-Bush et al. (1975) after 14 days. Changes reported here for the hippocampus 30 days after pCA agree with those reported by Sanders-Bush et al. (1975) 4 hours after treatment, by Neckers et al. (1975) 14 days after treatment and by Meek and Bertilsson (1975) 60 days after treatment. The levels of 5HT found 3 days after pCA treatment in this study are somewhat higher than those reported in the literature. Sanders-Bush et al. (1975) found that 5HT levels were decreased in the corpus striatum much more than reported in this study.

In pCA treated, tryptophan loaded animals the levels of endogenous 5HT in the mesencephalon-pons increased by 44% when the tryptophan load was administered 3 days after pCA treatment and by 53% when given 30 days after a pCA treatment. In the diencephalon these increases were 52% in animals treated 3 days previously with pCA and 64% in animals treated 30 days previously. These increases agree with those reported for whole brain by Fuller and Snoddy (1974).

In the hippocampus and corpus striatum the changes in pCA treated animals after a tryptophan load found here do not agree with the whole brain measurements of Fuller and Snoddy (1974). In the hippocampus there is an unexplainable reduction in 5HT in animals who were given a tryptophan load 3 days after pCA treatment. The levels of 5HT in animals who received a tryptophan load 3 days after pCA treatment are congruous with levels normally reported for the hippocampus after pCA alone. Perhaps differences in technique account for this. Consequently, the results noted after 3 days in pCA treated animals should be replicated before further speculation on the unexpected decrease in 5HT following a tryptophan load in these animals. Thirty days after pCA a tryptophan load resulted in an 87% increase in the level of 5HT. This increase is much greater than for whole brain (Fuller and Snoddy, 1974).

In the corpus striatum there was no change in the level of 5HT after a tryptophan load in animals who had been treated 3 days previously with pCA, but there was a 93% increase in levels of this amine in animals who were given a tryptophan load 30 days after pCA treatment.

It is evident from these results that there is marked regional variation in the effect of pCA on the ability to metabolize a tryptophan load. In the mesencephalon-pons and in the diencephalon at both 3 and 30 days after pCA the levels of 5HT increase 45 - 65% after a tryptophan load. Three days after pCA the levels return to 75% of control after the tryptophan load and in 30 day animals they return to control levels. Curzon and Marsden (1975) have shown that 30 minutes after a tryptophan load of 100 mg/kg, levels of 5HT increase 50% to 55% depending on the area of the brain analyzed. Thus the

increases noted above may reflect a normal increase in 5HT levels after a tryptophan load. In addition this percentage increase appears to be independent of the endogenous levels of 5HT present since in both groups studied the levels were decreased below normal while those reported by Curzon and Marsden (1975) were normal.

Gal et al. (1975) and Sanders-Bush et al. (1975) have suggested that tryptophan hydroxylase activity is inhibited initially after pCA, returns to near normal activity in animals 3 days after treatment but decreases thereafter and remains depressed for at least 14 days. The results reported here for the mesencephalon-pons and diencephalon show normal percentage increases in 5HT after a tryptophan load at both 3 and 30 days after a pCA treatment. These results suggest that the enzyme present in these areas is working at a normal rate at both time periods which supports those results found by Gal et al. (1975) and Sanders-Bush et al. (1975) for animals 3 days after pCA treatment. In the corpus striatum however, endogenous levels of 5HT were not increased in animals given a tryptophan load 3 days after pCA treatment while 30 days after pCA treatment 5HT levels increased by 90%. Although these results do not agree with those found by Gal et al. (1975) and Sanders-Bush et al (1975), the present work is based on an in vivo assay of tryptophan conversion to 5HT while the work of Gal et al. (1975) and Sanders-Bush et al. (1975) was based on in vitro assays.

Sekerke et al. (1975) have reported that pCA has different effects on tryptophan hydroxylase isolated from forebrain areas and from midbrain areas. Forebrain enzyme was depressed to a much greater extent than was the midbrain enzyme. This depressed state was maintained for at least

1 week. In the corpus striatum of animals who were treated 3 days previously with pCA, a tryptophan load does not alter the endogenous levels of 5HT at all which suggests that tryptophan hydroxylase activity is virtually blocked at this time. This does not agree with some workers' reports (Gal et al., 1975; Sanders-Bush et al., 1975) but does support the work of Sekerke et al. (1975). In animals who have been treated 30 days previously with pCA, however, endogenous levels of 5HT are increased by 90% following a tryptophan load. Similar increases in endogenous levels of 5HT occur in the hippocampus when tryptophan is administered 30 days after pCA.

Neckers et al. (1975) have reported that tryptophan hydroxylase activity in the corpus striatum and in the hippocampus is still significantly reduced for up to 60 days after treatment. The results reported here do not support this.

It appears from this work that pCA affects endogenous levels of 5HT in the diencephalon and mesencephalon-pons but does not affect the animals' ability to synthesize 5HT after a tryptophan load via existing tryptophan hydroxylase. This suggests that the decrease is due to some effect other than inhibition of tryptophan hydroxylase. One possible explanation is that there is a reduction in sites where 5HT is synthesized. In the corpus striatum on the other hand, tryptophan hydroxylase appears to be completely inhibited 3 days after pCA treatment but becomes active again 30 days after treatment. However, the levels of 5HT are not significantly reduced at 3 or 30 days after a pCA treatment. Perhaps 5HT is being supplied to this area via axonal transport or alternatively the uptake of tryptophan

into this area is prevented at this time by pCA treatment. Further area studies on the effects of pCA on the uptake of tryptophan should be conducted to clarify these results.

The hippocampus is the brain area most affected by pCA treatment. 5HT levels in this area are reduced by 58% 30 days after a single injection of pCA. After a tryptophan load, the levels are still reduced by 22% in animals who have been given pCA 30 days previously. In all other areas the levels of 5HT have returned to normal after a tryptophan load in animals who have been given pCA 30 days previously.

Further work is required to replicate the results in the hippocampus and corpus striatum. In addition isolation of tryptophan hydroxylase from all areas studied for in vitro analysis of activity is essential. Measurements should also be made at an intermediate time such as 14 days after pCA treatment and a lower dosage of pCA should be tested. Finally, turnover studies especially in the corpus striatum should be conducted to see if the 5HT half-life has increased in this area.

(6.) SUMMARY AND CONCLUSIONS

This study examined the effects of a single injection of pCA on the regional distribution in rat brain of DA, NA and 5HT. As the action of pCA has been thought to be specific for 5HT it was predicted that pCA would have no effect on the levels of DA and NA at either of the two different time periods studied whilst it would have a major effect on 5HT at both these time periods. Further since the action of pCA appears to be specifically centered in axonal terminal areas, it was also predicted that when a regional analysis was conducted a major effect would be seen in these areas compared to cell body areas.

One of the major effects of pCA is to decrease tryptophan hydroxylase activity. As pCA appears to be most active in terminal areas it was also predicted that in animals who were treated with pCA there would be less 5HT produced from a tryptophan load in these areas than in cell body areas.

Finally, since it is presently thought that the effects of pCA are long lasting, it was predicted that the observations made after 30 days would be the same as those made after 3 days.

1. As predicted pCA had no significant effect on the levels of DA or NA in any of the areas studied.
2. pCA significantly reduced the levels of 5HT in the mesencephalon-pons and diencephalon after both time periods. The levels in the hippocampus were reduced after 30 days while those in the corpus striatum were unchanged at either time period. The decrease in the levels of 5HT in the hippocampus 30 days after drug treatment was

much greater than other areas studied which was as predicted. The lack of effects in the corpus striatum was completely unexpected. Perhaps the corpus striatum receives 5HT from cell body areas via axoplasmic flow. This possibility needs further testing.

3. The effects of pCA on the animals' ability to metabolize a tryptophan load to 5HT were as follows:

The effects noted in the mesencephalon-pons and in the diencephalon were remarkably similar. In both areas the levels of 5HT increased similarly at both times studied, after a tryptophan load. The percentage increase in 5HT levels over endogenous levels of amine were as expected from the literature for control animals suggesting that the enzyme present was working at a normal rate.

At 30 days after pCA, 5HT in the hippocampus unexpectedly increased to greater levels over endogenous levels than in the mesencephalon-pons and diencephalon. In the corpus striatum 3 days after pCA treatment a tryptophan load does not change the endogenous levels of 5HT, while 30 days after pCA treatment a tryptophan load results in very large increases of 5HT similar to those for the hippocampus. Perhaps 3 days after pCA treatment, there is reduced uptake of tryptophan into the corpus striatum while at 30 days uptake has returned to normal. The greater percentage increases of 5HT over endogenous levels in the corpus striatum and hippocampus compared to the mesencephalon-pons and diencephalon could also be due to differences in uptake of tryptophan into these areas after pCA treatment.

4. In the mesencephalon-pons there was no difference in the levels of 5HT 3 days and 30 days after treatment. In the diencephalon there was a slight but statistically significant recovery in the levels of 5HT when levels obtained 30 days after treatment were compared to those obtained 3 days after treatment. This level was still significantly lower than control. In the corpus striatum there was still no statistically significant decrease in 5HT 30 days after pCA treatment. There was no difference in the effects of pCA treatment on a tryptophan load at 3 days and 30 days after treatment in the mesencephalon-pons and diencephalon areas. In the corpus striatum there was a remarkable difference in the effects of pCA treatment on a tryptophan load when the load was given 3 days after pCA treatment compared to 30 days after pCA treatment. It appears then from this experiment that the prolonged effects of this drug are area dependent.

5. The differences expected in terminal areas compared to cell body areas were not found. It appears rather that there is a remarkable similarity within the diencephalon and mesencephalon-pons and a difference from the hippocampus and corpus striatum especially in the animals' ability to handle a tryptophan load. When the effects of pCA alone are assessed, there are similar decreases noted in the mesencephalon-pons and diencephalon but a marked difference between the effects in the hippocampus compared to the corpus striatum. Therefore when examining the results obtained after pCA alone, there appears to be a regional variation instead of terminal areas versus cell body areas, or forebrain versus brainstem. Thus when assessing the effects of a drug on the levels of amine in the brain it is crucial to conduct regional analysis of these effects.

REFERENCES

- Anden, N.E., Dahlstrom, A., Fuxe, K., Larsson, K., Olson, L. and Ungerstedt, U. Ascending monoamine neurons to the telecephalon and diencephalon. *Acta physiol. Scand.* 67: 313-326, 1966.
- Ansell, G.B. and Beeson, M.F. A rapid and sensitive procedure for the combined assay of noradrenaline, dopamine and serotonin in a single brain sample. *Anal. Biochem.* 23: 196-206, 1968.
- Axelrod, J. O-methylation of epinephrine and other catechols in vitro and in vivo. *Science* 126: 400-401, 1957.
- Axelrod, J. Noradrenaline: Fate and control of its biosynthesis. *Science* 173: 598-606, 1971.
- Axelrod, J., Albers, W. and Clemente, C.D. Distribution of catechol-o-methyltransferase in the nervous system and other tissues. *J. Neurochem.* 5: 68-72, 1959.
- Axelrod, J. and Tomchick, R. Enzymatic o-methylation of epinephrine and other catechols. *J. Biol. Chem.* 233: 702-705, 1958.
- Barbeau, A. Role of dopamine in the nervous system. pp. 114-136. In "Monographs in Human Genetics", Vol. 6, ed. J. Francois, S. Karger, Basel, 1972.
- Barchas, J.D., Garanello, R.D., Stolk, J.M., Brodie, H.K.H. and Hamburg, D.A. Biogenic amines and behavior. pp. 235-329. In "Hormones and Behavior", ed. S. Levine, Academic Press, New York, 1972.
- Berger, B.D., Wise, C.D. and Stein, L. Norepinephrine: Reversal of anorexia in rats with lateral hypothalamic damage. *Science* 172: 281-284, 1971.
- Bertilsson, L., Koslow, S.H. and Costa, E. 5-hydroxytryptamine depletion in mesencephalic nuclei of rat brain following a single injection of p-chloroamphetamine. *Brain Res.* 91: 348-350, 1975.
- Bieger, D. and Hockman, C.H. On the physiology and pharmacology of cerebral dopamine neurons. pp. 215-325. In "Chemical Nervous System", ed. G.H. Hockman and D. Bieger, University Park Press, Baltimore, 1976.
- Bjorklund, A., Baumgarten, H.G. and Nobin, A. Chemical lesioning of central monoamine axons by means of 5,6-dihydroxytryptamine and 5,7-dihydroxytryptamine. *Adv. Biochem. Psychopharmacol.* 10: 13-33, 1974.

- Blaschko, H., Richter, D. and Scholssmann, H. The oxidation of adrenaline and other amines. *Biochem. J.* 31: 2187-2196, 1937.
- Boulton, A.A. Identification, distribution, metabolism and function of meta and para tyramine, phenylethylamine and tryptamine in brain. *Adv. Biochem. Psychopharmacol.* 15: 57-67, 1976.
- Bunney, W.E. and Davis, J.M. Norepinephrine in depressive reactions: Review. *Arch. Gen. Psychiatry* 13: 483-494, 1965.
- Butterworth, R.F., Landreville, F., Guitard, M. and Barbeau, A. A reliable method for the simultaneous estimation of dopamine, noradrenaline and serotonin in discrete areas of brain. *Clin. Biochem.* 8: 298-302, 1975.
- Carlsson, A., Falck, B. and Hillarp, N.A. Cellular localization of brain monoamines. *Acta physiol. scand.* 56: Suppl 196, 1-28, 1962.
- Chang, C.C. A sensitive method for spectrophotofluorometric assay of catecholamines. *Int. J. Neuropharmacol.* 3: 643-649, 1964.
- Chase, T.N. and Murphy, D.L. Serotonin and central nervous system function. *Anal. Rev. Pharmacol.* 13: 181-197, 1973.
- Chirigos, M.A., Greengard, P. and Udenfriend, S. Uptake of tyrosine by rat brain in vivo. *J. Biol. Chem.* 235: 2075-2079, 1960.
- Coppen, A., Prange, A.J., Whybrow, P.C. Methysergide in mania. *Lancet* ii: 338-340, 1969.
- Costa, E., Naimzada, K.M. and Revuelta, A. Effect of phenmetrazine, aminorex and (\pm) p-chloroamphetamine on the motor activity and turnover rate of brain catecholamines. *Br. J. Pharmacol.* 43: 570-579, 1971.
- Costa, E. and Revuelta, A. (-) -p- chloroamphetamine and serotonin turnover in rat brain. *Neuropharmacology* 11: 291-295, 1972.
- Cox, R.H. and Perhach, J.L. A sensitive, rapid and simple method for the simultaneous spectrophotofluorometric determination of norepinephrine, dopamine, 5-hydroxytryptamine and 5-hydroxy-indoleacetic acid in discrete areas of brain. *J. Neurochem.* 20: 1777-1780, 1973.
- Curzon, G. and Green, A.R. Rapid method for the determination of 5-hydroxytryptamine and 5-hydroxyindole acetic acid in small regions of rat brain. *Br. J. Pharmacol.* 39: 653-655, 1970.
- Curzon, G. and Marsden, C.A. Metabolism of a tryptophan load in the hypothalamus and other brain regions. *J. Neurochem.* 25: 251-256, 1975.

- Dahlstrom, A. and Fuxe, K. Evidence for the existence of monoamine-containing neurons in the central nervous system. I Demonstration of monoamines in the cell bodies of brain stem neurons. *Acta physiol. Scand.* 62: Suppl 232, 1-55, 1964.
- Dahlstrom, A. and Fuxe, K. Evidence for the existence of monoamine neurons in the central nervous system. II. Experimentally induced changes in the interneuronal amine levels of bulbospinal neuron systems. *Acta physiol. Scand.* 64: Suppl 247, 1-34, 1965.
- Deguchi, T. and Barchas, J.D. Regional distribution and developmental change of tryptophan hydroxylase activity in the rat brain. *J. Neurochem.* 19: 927-929, 1972.
- Dewhurst, W.G. New theory of cerebral amine function and its clinical application. *Nature* 218: 1130-1133, 1968.
- Dewhurst, W.G. Methysergide in mania. *Nature* 219: 506-507, 1968.
- Dewhurst, W.G. Amines and abnormal mood. *Proc. Roy. Soc. Med.* 62: 1102-1107, 1969.
- Dewhurst, W.G. and Marley, E. Action of sympathomimetic and allied amines on the central nervous system in the chicken. *Br. J. Pharmacol.* 25: 705-727, 1965.
- Donovan, B.T. *Mammalian Neuroendocrinology*. McGraw-Hill, London, 1970.
- Dring, L.G., Smith, R. and Williams, R.T. The metabolic fate of amphetamine in man and other species. *Biochem. J.* 116: 425-435, 1970.
- Falck, B., Hillary, N.A., Thieme, G. and Torp, A. Fluorescence of catecholamines and related compounds condensed with formaldehyde. *J. Histochem. Cytochem.* 10: 348-354, 1962.
- Fieve, R.R., Platman, S.R. and Fliess, J.L. A clinical trial of methysergide and lithium in mania. *Psychopharmacologia* 15: 425-429, 1969.
- Fuller, R.W. and Hines, C.W. Tissue levels of chloroamphetamines in rats and mice. *J. Pharm. Sci.* 56: 320-303, 1967.
- Fuller, R.W. and Hines, C.W. Inhibition by p-chloroamphetamine of the conversion of 5-hydroxytryptamine to 5-hydroxyindole acetic acid in rat brain. *J. Pharm. Pharmacol.* 22: 634-635, 1970.
- Fuller, R.W., Hines, C.W. and Mills, J. Lowering of brain serotonin level by chloramphetamine. *Biochem. Pharmacol.* 14: 483-488, 1965.

- Fuller, R.W. and Perry, K.W. Long-lasting depletion of brain serotonin by 4-chloroamphetamine in guinea pigs. *Brain Res.* 82: 383-385, 1974.
- Fuller, R.W., Perry, K.W. and Molloy, B.B. Reversible and irreversible phases of serotonin depletion by 4-chloroamphetamine. *Eur. J. Pharmacol.* 33: 119-124, 1975.
- Fuller, R.W. Schaffer, R.J., Roush, B.W., and Molloy, B.B. Drug disposition as a factor in the lowering of brain serotonin by chloroamphetamine in the rat. *Biochem. Pharmacol.* 21: 1413-1417, 1972.
- Fuller, R.W. and Snoddy, H.D. Long-term effects of 4-chloroamphetamine on brain 5-hydroxyindole metabolism in rats. *Neuropharmacology* 13: 85-90, 1974.
- Fuxe, C. and Jonsson, G. Further mapping of central 5-hydroxytryptamine neurons: Studies with the neurotoxic dihydroxytryptamines. *Adv. Biochem. Psychopharmacol.* 10: 1-12, 1974.
- Gal, E.M., Christiansen, P.A. and Yungler, L.M. Effect of p-chloroamphetamine on cerebral tryptophan-5-hydroxylase in vivo: A reexamination. *Neuropharmacology* 14: 31-39, 1975.
- Gessa, G.L. and Tagliamonte, A. Possible role of brain serotonin and dopamine in controlling male sexual behavior. *Adv. Biochem. Psychopharmacol.* 11: 217-228, 1974.
- Goodman, L.S. and Gilman, A. *The Pharmacological Basis of Therapeutics* 4th Ed. MacMillan and Company, London, 1970.
- Grahame-Smith, D.G. Tryptophan hydroxylation in brain. *Biochem. Biophys. Res. Commun.* 16: 586-592, 1964.
- Grahame-Smith, D.G. The biosynthesis of 5-hydroxytryptamine in brain. *Biochem. J.* 106: 351-360, 1967.
- Green, A.R. and Grahame-Smith, D.G. 5-hydroxytryptamine and other indoles in the central nervous system. pp. 169-245. In "Handbook of Psychopharmacology" Vol. 3, ed. L.L. Iversen, S.D. Iversen and S.H. Snyder. Plenum Press, New York, 1975.
- Grof, P. and Foley, P. The superiority of lithium over methysergide in treating manic patients. *Am. J. Psychiatry* 127: 1573-1574, 1971.
- Harvey, J.A., McMaster, S.E. and Yonger, L.M. p-chloroamphetamine: selective neurotoxic action in brain. *Science* 187: 841-843, 1975.
- Haskovec, L. Methysergide in mania. *Lancetii*: 902, 1969.

- Haskovec, L. and Soucek, K. Trial of methysergide in mania. *Nature*, 219: 507-508, 1968.
- Haubrich, D.R. and Denzer, J.S. Simultaneous extraction and fluorometric measurement of brain serotonin, catecholamines, 5-hydroxy-indole acetic acid and homovanillic acid. *Anal. Biochem.* 55: 306-312, 1973.
- Hutchinson, R.R. and Renfrew, J.W. Modification of eating and drinking. Interaction between chemical agent, deprivation state and site of stimulation. *J. Comp. physiol. Psychol.* 63: 408-416, 1967.
- Iversen, L.L. Role of transmitter uptake mechanisms in synaptic neurotransmission. *Br. J. Pharmacol.* 41: 571-591, 1971.
- Jouvet, M. Insomnia and decrease of cerebral 5-hydroxytryptamine after destruction of the raphe system in the cat. *Adv. Pharmacol.* 6: 269-275, 1968.
- Jouvet, M. Monoaminergic regulation of the sleep-waking cycle in the cat. pp. 499-509. In "The Neurosciences: Third Study Program", ed. F.O. Schmitt and F.G. Worden, M.T. Press, Cambridge, Massachusetts, 1974.
- Kariya, T. and Aprison, M.H. Microdetermination of norepinephrine, 3,4-dihydroxyphenylethylamine and 5-hydroxytryptamine from single extracts of specific rat brain areas. *Anal. Biochem.* 31: 102-113, 1969.
- Kline, N.A., Sacks, W. and Simpson, G.M. Further studies on: One day treatment of depression with 5HTP. *Am. J. Psychiatry* 121: 379-381, 1964.
- Knapp, S., Mandell, A.J. and Geyer, M.A. Effects of amphetamines on regional tryptophan hydroxylase activity and synaptosomal conversion of tryptophan to 5-hydroxytryptamine in rat brain. *J. Pharmacol. Exp. Ther.* 189: 676-689, 1974.
- Knott, P.J. and Curzon, G. Effect of increased rat brain tryptophan on 5-hydroxytryptamine and 5-hydroxyindole acetic acid in the hypothalamus and other brain regions. *J. Neurochem.* 22: 1065-1071, 1974.
- Koe, B.K. and Corkey, R.F. Inhibition of rat brain tryptophan hydroxylation with p-chloroamphetamine. *Biochem. Pharmacol.* 25: 31-35, 1976.
- Koe, B.K. and Weissman, A. p-chlorophenylalanine: A specific depletion of brain serotonin. *J. Pharmacol. Exp. Ther.* 154: 499-516, 1966.
- Lapin, I.P. and Oxenkrug, G.F. Intensification of the central serotonergic processes as a possible determinant of the thymoleptic effect. *Lancet* i: 132-136, 1969.

- Laverty, R. and Taylor, K.M. The fluorometric assay of catecholamines and related compounds: Improvements and extension to the hydroxyindole technique. *Anal. Biochem.* 22: 269-279, 1968.
- Leonard, B.E. Acute and chronic effects of 4-chloroamphetamine on monoamine metabolism in the rat brain. *Psychopharmacologia* 46: 11-18, 1976.
- Lindvall, O. and Bjorklund, A. The organization of the ascending catecholamine neuron systems in the rat brain. *Acta physiol. Scand. Suppl* 412, 1-48, 1974.
- Lovenberg, W., Jequier, E. and Sjoerdsma, A. Tryptophan hydroxylation: Measurement in pineal gland, brainstem and carcinoid tumor. *Science* 155: 217-219, 1967.
- Lovenberg, W., Weissbach, M. and Udenfriend, S. Aromatic L-amino acid decarboxylase. *J. Biol. Chem.* 237: 89-93, 1962.
- Maickel, R.P., Cox, R.H., Saillant, J. and Miller, F.P. A method for the determination of serotonin and norepinephrine in discrete areas of rat brain. *Int. J. Neuropharmacol.* 7: 275-281, 1968.
- Mandell, A.J. and Segal, D.S. The Psychobiology of dopamine and the methylated indoleamines with particular reference to psychiatry. pp. 89-112. In "Biological Psychiatry", ed. J. Mendels, John Wiley and Sons, New York, 1973.
- Marley, E. and Stephenson, J.D. Central actions of catecholamines. pp. 463-437. In "Handbook of Experimental Pharmacology", ed. H. Blaschko and E. Muscholl, Springer-Verlag, Berlin, 1972.
- Massari, V.J. and Sanders-Bush, E. Synaptosomal uptake and levels of serotonin in rat brain areas after p-chloroamphetamine or B-9 lesions. *Eur. J. Pharmacol.* 33: 419-422, 1975.
- McCabe, M.S., Reich, T. and Winokur, G. Methysergide as a treatment for mania. *Am. J. Psychiatry* 127: 354-356, 1970.
- McGeer, P.L., Bagchi, S.P. and McGeer, E.G. Subcellular localization of tyrosine hydroxylase in beef caudate nucleus. *Life Sci.* 4: 1859-1867, 1965.
- McNamee, H.B., Le Poidevin, D. and Naylor, G.J. Methysergide in mania: A double-blind comparison with thioridazine. *Psychol. Med.* 2: 66-69, 1972.
- Mendels, J.L. and Frazer, A. Brain biogenic amine depletion and mood. *Arch. Gen. Psychiatry* 30: 447-451, 1974.
- Meek, J.L. and Bertilsson, L. Comparison of the effects of lesion in the "B9" cell body group and p-chloroamphetamine on tryptophan hydroxylase and 5-hydroxytryptamine in rat brain nuclei. *Brain Res.* 100: 140-144, 1975.

- Miller, F.P., Cox, R.H., Snodgrass, W.R. and Naichel, R.P. Comparative effects of p-chlorophenylalanine, o-chloroamphetamine and p-chloro-N-methylamphetamine on rat brain norepinephrine, serotonin and 5-hydroxyindole-3-acetic acid. *Biochem. Pharmacol.* 19: 435-442, 1970.
- Miller, K.W., Sanders-Bush, E. and Dingell, J.V. p-chloroamphetamine - species differences in the rate of disappearance and lowering of cerebral serotonin. *Biochem. Pharmacol.* 20: 500-503, 1971.
- Modigh, K. and Svensson, T.H. On the role of central nervous system catecholamines and 5-hydroxytryptamine in the nialamide-induced behavioral syndrome. *Br. J. Pharmacol.* 46: 32-45, 1972.
- Montagu, K.A. Catechol compounds in rat tissues and in brains of different animals. *Nature* 180: 244-245, 1957.
- Morgan, D. Lofstrandh, S. and Costa, E. Amphetamine analogues and brain amines. *Life Sci.* 11: 83-96, 1972.
- Nagatsu, T. *Biochemistry of catecholamines: The biochemical method.* University Park Press, Tokyo, 1973.
- Nagatsu, T. and Nagatsu, I. Subcellular distribution of tyrosine hydroxylase and monoamine oxidase in the bovine caudate nucleus. *Experientia* 26: 722-723, 1970.
- Neckers, L.M., Bertilsson, L., Koslow, S.H. and Meek, J.L. Reduction of tryptophan hydroxylase activity and 5-hydroxytryptamine concentration in certain rat brain nuclei after p-chloroamphetamine. *J. Pharmacol. Exp. Ther.* 196: 333-338, 1975.
- Neff, N.H., Yang, H.Y.T., Goridis, C. and Bialek, D. The metabolism of indolealkylamines by Type A and B monoamine oxidase of brain. *Adv. Biochem. Psychopharmacol.* 11: 51-58, 1974.
- Pletscher, A., Bartholini, G., Bruderer, H., Burkard, W.P. and Gey, K.F. Chlorinated arylalkylamines affecting the cerebral metabolism of 5-hydroxytryptamine. *J. Pharmacol. Exp. Ther.* 145: 344-350, 1964.
- van Praag, H.M., Korf, J. and Van Woudenberg, F. Investigation into the possible influence of chlorinated amphetamine derivatives on 5-hydroxytryptamine synthesis in man. *Psychopharmacologia* 18: 412-420, 1970.
- van Praag, H.M., Schut, T., Bosma, E. and Vanden Bergh, R. A comparative study of the therapeutic effects of some 4-chlorinated amphetamine derivatives in depressed patients. *Psychopharmacologia* 20: 66-76, 1971.

- de Robertis, E., Rodriguez de Lores Arniag, G. Structural components of the synaptic region. pp. 365-392. In "Handbook of Neurochemistry" Vol. 11, ed. A. Lajtha, Plenum, New York, 1969.
- Saavedra, J.M., Brownstein, M. and Axelrod, J. A specific and sensitive enzymatic-isotopic microassay for serotonin in tissues. J. Pharmacol. Exp. Ther. 186: 508-515, 1973.
- Sanders-Bush, E. and Sulser, F. p-chloroamphetamine: In vivo investigations on the mechanism of action of the selective depletion of cerebral serotonin. J. Pharmacol. Exp. Ther. 175: 419-426, 1970.
- Sanders-Bush, E., Bushing, J.A. and Sulser, F. p-chloroamphetamine - inhibition of cerebral tryptophan hydroxylase. Biochem. Pharmacol. 21: 1501-1510, 1972(a).
- Sanders-Bush, E., Bushing, J.A. and Sulser, F. Long-term effects of p-chloroamphetamine on tryptophan hydroxylase activity and on the levels of 5-hydroxytryptamine and 5-hydroxyindole acetic acid in brain. Eur. J. Pharmacol. 20: 385-388, 1972(b).
- Sanders-Bush, E., Bushing, J.A. and Sulser, F. Long-term effects of p-chloroamphetamine and related drugs on central serotonergic mechanisms. J. Pharmacol. Exp. Ther. 192: 33-41, 1975.
- Schildkraut, J.J. The catecholamine hypothesis of affective disorders: a review of supporting evidence. Am. J. Psychiatry 122: 509-522, 1965.
- Schildkraut, J.J. Depressions and biogenic amines. pp. 460-487. In "American Handbook of Psychiatry", ed. D.A. Hamburg and K.H. Brodie, Basic Books, New York 1975.
- Schnaitman, C., Erwin, U.G. and Greenawalt, J.W. Submitochondrial localization of monoamine oxidase. J. Cell Biol. 34: 719-735, 1967.
- Sekerke, H.J., Smith, H.E., Bushing, J.A. and Sanders-Bush, E. Correlation between brain levels and biochemical effects of the optical isomers of p-chloroamphetamine. J. Pharmacol. Exp. Ther. 193: 835-844, 1975.
- Serry, D. Methysergide in mania. Lancet : 417, 1969.
- Shellenberger, M.K. and Gordon, J.H. A rapid simplified procedure for simultaneous assay of norepinephrine, dopamine and 5-hydroxytryptamine from discrete brain areas. Anal. Biochem. 39: 356-372, 1971.
- Sims, K.L. Biochemical characteristics of mammalian brain 5-hydroxytryptamine decarboxylase activity. Adv. Biochem. Psychopharmacol. 11: 43-50, 1974.

- Snyder, S.H., Axelrod, J. and Zweig, M. A sensitive and specific fluorescence assay for tissue serotonin. *Biochem. Pharmacol.* 14: 831-835, 1965.
- Spector, S., Gordon, R., Sjoerdsma, A. and Udenfriend, S. End-product inhibition of tyrosine hydroxylase as a possible mechanism for the regulation of norepinephrine synthesis. *Mol. Pharmacol.* 3: 540-555, 1967.
- Stein, L. and Wise, C.D. Release of hypothalamic norepinephrine by rewarding electrical stimulation or amphetamine in the unanesthetized rat. *Fed. Proc.* 26: 651, 1967.
- Stein, L. and Wise, C.D. Serotonin and behavioral inhibition. *Adv. Biochem. Psychopharm.* 11: 281-291, 1974.
- Stjarne, L. Studies of noradrenaline biosynthesis in nerve tissue. *Acta physiol. Scand.* 67: 441-454, 1966.
- Strada, S.J., Sanders-Bush, E. and Sulser, F. p-chloroamphetamine temporal relationship between psychomotor stimulation and metabolism of brain norepinephrine. *Biochem. Pharmacol.* 19: 2621-2629, 1970.
- Strada, S.J. and Sulser, F. Comparative effects of p-chloroamphetamine and amphetamine on metabolism and in vivo release of 3-norepinephrine in the hypothalamus. *Eur. J. Pharmacol.* 15: 45-51, 1971.
- Tabakoff, B., Moses, F., Philips, S.R. and Boulton, A.A. Effects of tranylcypromine and pargyline on brain tryptamine. *Experientia*, in press 1977.
- Twarog, B.M. and Page, I.H. Serotonin content of some mammalian tissues and urine and a method for its determination. *Am. J. Physiol.* 175: 157-161, 1953.
- Udenfriend, S. Biosynthesis and release of catecholamines. p.13. In "Mechanism of Release of Biogenic Amines", Proceedings of an international Wenner-Gren symposium, Stockholm, Pergamon Press, London, 1966.
- Ungerstedt, U. Stereotoxic mapping of the monoamine pathways in the rat brain. *Acta physiol. Scand. Suppl* 367: 1-48, 1971.
- Ungerstedt, U. Brain dopamine neurons and behavior. pp. 695-703. In "The Neurosciences: Third Study Program". ed. F.O. Schmitt and F.G. Worden, The MIT Press, Cambridge, Massachusetts, 1974.
- Versteeg, D.H.G. and Wurtman, J. Synthesis and release of monoamine neurotransmitters: Regulating mechanisms. pp. 201-234. In "Molecular and Functional Neurobiology". ed. W.H. Gispen, Elsevier Scientific Publishing Company, New York, 1976.

- Verster, J.P. Preliminary report on the treatment of mentally disordered patients by intrathecally administered phenothiazine drugs and an antiserotonin substance. S. Af. Med. J. 37: 1086-1087, 1963.
- Vogt, M. The concentration of sympathin in different parts of the central nervous system under normal conditions and after the administration of drugs. J. Physiol. 123: 451-481, 1954.
- Whittaker, V.P. The synaptosome. pp. 327-364. In "Handbook of Neurochemistry", Vol. 11, ed. A. Lajtha, Plenum, New York 1969.
- Whittaker, V.P. The biochemistry of synaptic transmission. Naturwissenschaften 60: 281-389, 1973.
- Wong, D.T., Van Frank, R.M., Horng, J.S. and Fuller R.W. Accumulation of amphetamine and p-chloroamphetamine into synaptosomes of rat brain. J. Pharm. Pharmac. 24: 171-173, 1972.
- Wong, D.T., Horng, J.S. and Fuller, R.W. Kinetics of serotonin accumulation into synaptosomes of rat brain - effects of amphetamine and chloroamphetamine. Biochem. Pharmacol. 22: 311-322, 1973.
- Wurzbürger, R.J. and Musacchio, J.M. Subcellular distribution and aggregation of bovine adrenal tyrosine hydroxylase. J. Pharmacol. Exp. Ther. 177: 155-168, 1971.

APPENDIX A

Chemicals

n-Butanol (Spectrofluorometric grade)	Fisher Scientific
Ethanol (absolute)	U.S. Industrial Chemicals
n-Heptane	Mallinckrodt
Hydrochloric Acid	Baker Chemicals
Glacial Acetic Acid	Fisher Scientific
Boric Acid	Fisher Scientific
Sodium Hydroxide	BDH Chemicals
Sodium Chloride	McArthur Chemicals
Sodium Sulfite	Baker Chemicals
Sodium Acetate	Fisher Scientific
Disodium (Ethylenedinitrilo) Tetraacetate (EDTA)	Baker Chemicals
Alumina (Acidic Brockmann No. 1)	Terochem Laboratories
Iodine	Baker Chemicals
Serotonin creatinine sulfate complex	Sigma Chemicals
Dopamine HCL	Sigma Chemicals
Noradrenaline HCL	Sigma Chemicals
o-phthalaldehyde	Sigma Chemicals

APPENDIX BEquipment

Expandomatic pH Meter	Beckman Instruments
Aminco Bowman Spectrophotofluorometer	American Instruments
GLC-1 Bench Top Centrifuge	Sorvall Instruments
Evapo-Mix Shaker	Buchler Instruments
Stir-R Tissue Homogenizer	Tri-R Instruments
Mixer	Scientific Products
Revco Ultra Low Temp Freezer	Revco Industrial Products

APPENDIX CSolutions

Glass distilled water was used throughout in the preparation of the following reagents. The water was prepared in the laboratory and was run through a demineralizing column just before use.

1. Acidified Butanol: 0.85 ml of concentrated HCl was added to one liter of n-butanol.
2. EDTA reagent: a 0.1M EDTA solution was prepared by dissolving 37.2 gm of disodium (ethylenedinitrilo) tetraacetate in 1 liter of 1M sodium acetate. 1M sodium acetate was prepared by dissolving 136 gm of sodium acetate in 1 liter of distilled water. This reagent was stored in 2 ml aliquots at -20°C.
3. Iodine reagent: a 0.1M iodine solution was prepared by dissolving 1.27 gm of iodine in 100 ml of absolute ethanol.
4. Alkaline sulfite reagent: 0.25 gm of sodium sulfite was dissolved in 1 ml of distilled water and alkalinized with 9 ml of 5N NaOH. This reagent was prepared just before use.
5. 2M sodium acetate was prepared by dissolving 27.2 gm of sodium acetate in 100 ml of distilled water.
6. 0.35M Borate buffer (pH 10) was prepared by dissolving 3.14 gm of boric acid in 100 ml of distilled water followed by the addition of 5.5 ml of 10N NaOH. The pH was adjusted to 10, if necessary, with 10N NaOH.
7. o-phthalaldehyde reagent: a 0.01% solution of o-phthalaldehyde was prepared by dissolving 10 mg of o-phthalaldehyde in 100 ml of 10N HCl. This reagent was prepared just before use.
8. Standards were prepared by dissolving 0.5 mg of the amine based on weight of the salt in 1 ml of 0.01N HCl.

Levels of amines obtained from control animals after 3 days. Values are reported in $\mu\text{g/gm}$. Each sample is the pool of two brains. Means \pm standard deviations (S.D.) are reported for each group. N.D. = non-detectable.

	NA	DA	5HT
Mesencephalon-pons			
Sample 1.	.605	.150	.805
Sample 2.	.450	.111	.678
Sample 3.	.401	.185	.895
Sample 4.	.520	.146	.995
Mean \pm S.D.	.494 \pm .089	.148 \pm .030	.843 \pm .135
Diencephalon			
Sample 1.	.947	.251	.681
Sample 2.	.990	.300	1.000
Sample 3.	.613	.347	.863
Sample 4.	.757	.230	.547
Mean \pm S.D.	.827 \pm .175	.282 \pm .052	.773 \pm .199
Hippocampus			
Sample 1.	.354	N.D.	.233
Sample 2.	.263	N.D.	.563
Sample 3.	.315	N.D.	.375
Sample 4.	.321	N.D.	.315
Mean \pm S.D.	.313 \pm .038		.371 \pm .140
Corpus striatum			
Sample 1.	.172	6.875	.422
Sample 2.	.307	11.540	.371
Sample 3.	.236	10.111	.458
Sample 4.	.210	10.339	.548
Mean \pm S.D.	.231 \pm .057	9.716 \pm 1.995	.450 \pm .075

Levels of amines obtained from control animals after 30 days. Values are reported in $\mu\text{g/gm}$. Each sample is the pool of two brains. Means \pm standard deviations (S.D.) are reported for each group. N.D. = non-detectable.

	NA	DA	5HT
Mesencephalon-pons			
Sample 1.	.484	.191	.840
Sample 2.	.467	.229	1.124
Sample 3.	.454	.264	.810
Sample 4.	.512	.453	.828
Mean \pm S.D.	.479 \pm .025	.284 \pm .116	.901 \pm .150
Diencephalon			
Sample 1.	.926	.311	.896
Sample 2.	.687	.306	.836
Sample 3.	.863	.246	.738
Sample 4.	.705	.271	.729
Mean \pm S.D.	.795 \pm .118	.283 \pm .031	.800 \pm .08
Hippocampus			
Sample 1.	.373	N.D.	.450
Sample 2.	.265	N.D.	.353
Sample 3.	.418	N.D.	.276
Sample 4.	.393	N.D.	.434
Mean \pm S.D.	.362 \pm .068		.378 \pm .080
Corpus striatum			
Sample 1.	.214	14.871	.457
Sample 2.	.325	9.843	.675
Sample 3.	.309	9.704	.370
Mean \pm S.D.	.283 \pm .06	11.473 \pm 2.944	.501 \pm .157

Levels of amines obtained from tryptophan loaded animals after 3 days. Values are reported in $\mu\text{g/gm}$. Each sample is the pool of two brains. Means \pm standard deviations (S.D.) are reported for each group. N.D. = non-detectable.

	NA	DA	5HT
Mesencephalon-pons			
Sample 1.	.450	.100	1.294
Sample 2.	.546	.202	1.066
Sample 3.	.751	.219	1.134
Sample 4.	.538	.210	.871
Mean \pm S.D.	.571 \pm .217	.182 \pm .055	1.091 \pm .175
Diencephalon			
Sample 1.	.663	.699	1.113
Sample 2.	.665	.335	.886
Sample 3.	.985	.496	.891
Sample 4.	.815	.486	.795
Mean \pm S.D.	.782 \pm .153	.504 \pm .149	.921 \pm .135
Hippocampus			
Sample 1.	.346	N.D.	.519
Sample 2.	.401	N.D.	.574
Sample 3.	.341	N.D.	.681
Mean \pm S.D.	.363 \pm .033		.591 \pm .082
Corpus striatum			
Sample 1.	.257	10.843	.600
Sample 2.	.324	7.892	.676
Sample 3.	.500	12.073	.634
Mean \pm S.D.	.360 \pm .126	10.269 \pm 2.149	.637 \pm .038

Levels of amines obtained from tryptophan loaded animals after 30 days. Values are reported in $\mu\text{g/gm}$. Each sample is the pool of two brains. Means \pm standard deviations (S.D.) are reported for each group. N.D. = non-detectable.

	NA	DA	5HT
Mesencephalon-pons			
Sample 1.	.443	.115	1.404
Sample 2.	.481	.222	1.407
Sample 3.	.538	.210	.910
Mean \pm S.D.	.487 \pm .049	.182 \pm .059	1.240 \pm .286
Diencephalon			
Sample 1.	.706	.230	1.405
Sample 2.	.719	.404	.904
Sample 3.	.815	.486	.924
Mean \pm S.D.	.747 \pm .059	.373 \pm .131	1.078 \pm .284
Hippocampus			
Sample 1.	.330	N.D.	.725
Sample 2.	.263	N.D.	.758
Sample 3.	.250	N.D.	.571
Mean \pm S.D.	.281 \pm .043		.685 \pm .100
Corpus striatum			
Sample 1.	.292	10.333	.875
Sample 2.	.200	15.541	1.176
Sample 3.	.200	8.325	.488
Mean \pm S.D.	.231 \pm .053	11.400 \pm 3.724	.846 \pm .345

Levels of amines obtained from pCA treated animals after 3 days. Values are reported in $\mu\text{g/gm}$. Each sample is the pool of two brains. Means \pm standard deviations (S.D.) are reported for each group. N.D. = non-detectable.

	NA	DA	5HT
Mesencephalon-pons			
Sample 1.	.418	.144	.314
Sample 2.	.472	.112	.551
Sample 3.	.528	.130	.559
Sample 4.	.542	.112	.341
Mean \pm S.D.	.490 \pm .057	.125 \pm .015	.441 \pm .131
Diencephalon			
Sample 1.	.689	.227	.303
Sample 2.	.733	.298	.481
Sample 3.	.857	.364	.443
Sample 4.	.816	.202	.316
Mean \pm S.D.	.774 \pm .077	.273 \pm .073	.386 \pm .089
Hippocampus			
Sample 1.	.339	N.D.	.125
Sample 2.	.259	N.D.	.306
Sample 3.	.268	N.D.	.278
Sample 4.	.284	N.D.	.239
Mean \pm S.D.	.288 \pm .035		.237 \pm .079
Corpus striatum			
Sample 1.	.269	11.104	.149
Sample 2.	.236	8.583	.583
Sample 3.	.250	8.828	.297
Sample 4.	.218	5.974	.500
Mean \pm S.D.	.243 \pm .021	8.622 \pm 2.09	.382 \pm .196

Levels of amines obtained from pCA treated animals after 30 days. Values are reported in $\mu\text{g/gm}$. Each sample is the pool of two brains. Means \pm standard deviations (S.D.) are reported for each group. N.D. = non-detectable.

	NA	DA	5HT
Mesencephalon-pons			
Sample 1.	.518	.147	.624
Sample 2.	.492	.230	.481
Sample 3.	.404	.144	.505
Sample 4.	.510	.163	.540
Mean \pm S.D.	.481 \pm .053	.171 \pm .040	.537 \pm .063
Diencephalon			
Sample 1.	.902	.404	.515
Sample 2.	.838	.351	.466
Sample 3.	.786	.214	.508
Sample 4.	.789	.188	.586
Mean \pm S.D.	.829 \pm .054	.289 \pm .105	.519 \pm .05
Hippocampus			
Sample 1.	.240	N.D.	.115
Sample 2.	.312	N.D.	.256
Sample 3.	.303	N.D.	.128
Sample 4.	.355	N.D.	.132
Mean \pm S.D.	.303 \pm .047		.158 \pm .065
Corpus striatum			
Sample 1.	.268	10.207	.378
Sample 2.	.257	12.829	.343
Sample 3.	.254	11.407	.220
Sample 4.	.333	12.597	.472
Mean \pm S.D.	.278 \pm .037	11.760 \pm 1.21	.353 \pm .104

Levels of amines obtained from pCA treated plus tryptophan loaded animals after 3 days. Values are reported in $\mu\text{g/gm}$. Each sample is the pool of two brains. Means \pm standard deviations (S.D.) are reported for each group. N.D. = non-detectable.

	NA	DA	5HT
Mesencephalon-pons			
Sample 1.	.576	.124	.486
Sample 2.	.472	.157	.556
Sample 3.	.594	.120	.641
Sample 4.	.569	.133	.856
Mean \pm S.D.	.553 \pm .055	.133 \pm .017	.635 \pm .161
Diencephalon			
Sample 1.	.734	.237	.554
Sample 2.	.831	.331	.500
Sample 3.	.820	.258	.578
Sample 4.	.797	.301	.724
Mean \pm S.D.	.795 \pm .043	.282 \pm .042	.589 \pm .095
Hippocampus			
Sample 1.	.363	N.D.	.077
Sample 2.	.228	N.D.	.185
Sample 3.	.235	N.D.	.194
Sample 4.	.298	N.D.	.191
Mean \pm S.D.	.281 \pm .063		.162 \pm .057
Corpus striatum			
Sample 1.	.306	10.081	.145
Sample 2.	.187	10.107	.360
Sample 3.	.264	10.264	.486
Sample 4.	.240	9.200	.573
Mean \pm S.D.	.249 \pm .049	9.913 \pm .482	.391 \pm .185

Levels of amines obtained from pCA treated plus tryptophan loaded animals after 30 days. Levels are reported in $\mu\text{g/gm}$. Each sample is the pool of two brains. Means \pm standard deviations (S.D.) are reported for each group. N.D. = non-detectable.

	NA	DA	5HT
Mesencephalon-pons			
Sample 1.	.753	.141	.755
Sample 2.	.491	.156	.879
Sample 3.	.466	.130	.829
Sample 4.	.503	.203	.824
Mean \pm S.D.	.553 \pm .134	.157 \pm .032	.822 \pm .051
Diencephalon			
Sample 1.	.792	.388	1.011
Sample 2.	.661	.332	.712
Sample 3.	.815	.272	.834
Sample 4.	.847	.427	.847
Mean \pm S.D.	.779 \pm .082	.355 \pm .067	.851 \pm .123
Hippocampus			
Sample 1.	.462	N.D.	.333
Sample 2.	.310	N.D.	.345
Sample 3.	.258	N.D.	.227
Sample 4.		N.D.	.280
Mean \pm S.D.	.343 \pm .106		.296 \pm .054
Corpus striatum			
Sample 1.	.210	11.226	.532
Sample 2.			.772
Sample 3.	.375	10.038	.650
Sample 4.	.365	14.154	.769
Mean \pm S.D.	.317 \pm .092	11.806 \pm 2.118	.681 \pm .114

APPENDIX L

Dopamine

Means and standard deviations in $\mu\text{g/gm}$ of DA in the different areas of the rat brain. pCA was administered 3 days or 30 days before death at 10 mg/kg. Tryptophan was administered 1 hour before death at 100 mg/kg. Controls received injections of vehicle at equivalent times. Sample size is shown in brackets. N.D. = non-detectable.

	Control	Tryptophan load	pCA treated	pCA treated plus tryptophan load
Mesencephalon-pons				
3 day	.148 \pm .030 (4)	.182 \pm .055 (4)	.125 \pm .015 (4)	.133 \pm .017 (4)
30 day	.284 \pm .116 (4)	.182 \pm .059 (3)	.171 \pm .040 (4)	.157 \pm .032 (4)
Diencephalon				
3 day	.282 \pm .052 (4)	.504 \pm .149 (4)	.273 \pm .073 (4)	.282 \pm .042 (4)
30 day	.283 \pm .031 (4)	.373 \pm .131 (3)	.289 \pm .105 (4)	.355 \pm .067 (4)
Hippocampus				
3 day	N.D.	N.D.	N.D.	N.D.
30 day	N.D.	N.D.	N.D.	N.D.
Corpus striatum				
3 day	9.716 \pm 1.995 (4)	10.269 \pm 2.149 (3)	8.622 \pm 2.09 (4)	9.913 \pm .482 (4)
30 day	11.473 \pm 2.944 (3)	11.400 \pm 3.724 (3)	11.76 \pm 1.21 (4)	11.806 \pm 2.118 (3)

Means and standard deviations in $\mu\text{g/gm}$ of NA in the different areas of the rat brain. pCA was administered 3 days or 30 days before death at 10 mg/kg. Tryptophan was administered 1 hour before death at 100 mg/kg. Controls received injections of vehicle at equivalent times. Sample size is shown in brackets.

	Control	Tryptophan load	pCA treated	pCA treated plus tryptophan load
Mesencephalon-pons				
3 day	.494 \pm .089 (4)	.571 \pm .127 (4)	.490 \pm .057 (4)	.553 \pm .055 (4)
30 day	.479 \pm .025 (4)	.487 \pm .049 (3)	.481 \pm .053 (4)	.553 \pm .134 (4)
Diencephalon				
3 day	.827 \pm .175 (4)	.774 \pm .077 (4)	.795 \pm .077 (4)	.795 \pm .043 (4)
30 day	.795 \pm .118 (4)	.747 \pm .059 (3)	.829 \pm .054 (4)	.779 \pm .082 (4)
Hippocampus				
3 day	.313 \pm .038 (4)	.363 \pm .033 (3)	.288 \pm .035 (4)	.281 \pm .063 (4)
30 day	.362 \pm .068 (4)	.281 \pm .043 (3)	.303 \pm .047 (4)	.343 \pm .106 (3)
Corpus striatum				
3 day	.231 \pm .057 (4)	.360 \pm .126 (3)	.243 \pm .021 (4)	.249 \pm .049 (4)
30 day	.283 \pm .06 (4)	.231 \pm .053 (3)	.278 \pm .037 (4)	.317 \pm .092 (3)

APPENDIX N

5-Hydroxytryptamine

Means and standard deviations in $\mu\text{g/gm}$ of 5HT in the different areas of the rat brain. pCA was administered 3 days or 30 days before death at 10 mg/kg. Tryptophan was administered 1 hour before death at 100 mg/kg. Controls received injections of vehicle at equivalent times. Sample size is shown in brackets.

	Control	Tryptophan load	pCA treated	pCA treated plus tryptophan load
Mesencephalon-pons				
3 day	.834 \pm .135 (4)	1.091 \pm .175 (4)	.441 \pm .131 (4)	.635 \pm .161 (4)
30 day	.901 \pm .149 (4)	1.240 \pm .386 (3)	.537 \pm .063 (4)	.822 \pm .051 (4)
Diencephalon				
3 day	.773 \pm .199 (4)	.921 \pm .135 (4)	.386 \pm .089 (4)	.589 \pm .095 (4)
30 day	.800 \pm .080 (4)	1.078 \pm .284 (3)	.519 \pm .050 (4)	.851 \pm .123 (4)
Hippocampus				
3 day	.371 \pm .140 (4)	.591 \pm .082 (3)	.237 \pm .079 (4)	.162 \pm .057 (4)
30 day	.378 \pm .080 (4)	.685 \pm .100 (3)	.158 \pm .065 (4)	.296 \pm .054 (4)
Corpus striatum				
3 day	.450 \pm .075 (4)	.637 \pm .038 (3)	.382 \pm .196 (4)	.391 \pm .185 (4)
30 day	.501 \pm .157 (3)	.846 \pm .345 (3)	.353 \pm .104 (4)	.681 \pm .114 (4)

B30186